Institut für Lebensmittelsicherheit und -hygiene
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. Roger Stephan

Prevalence and characteristics of *Arcobacter butzleri* - a potential food borne pathogen - in fecal samples, on carcasses and in retail meat of cattle, pig and poultry in Switzerland

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Sibille Keller

Tierärztin

von Meggen LU

Sibylle Räber

Tierärztin

von Benzenschwil AG

genehmigt auf Antrag von

Prof. Dr. Roger Stephan, Referent

Prof. Dr. Kurt Houf, Korreferent

Zürich 2005

Sibille Keller
Für meine Eltern
Margrith und Peter

Sibylle Räber
Für meine Eltern
Kathrin und Roland

Content

1 Summary

Recent findings suggest, that arcobacters, especially *A. butzleri*, may be involved in human enteric diseases. There is evidence, that livestock animals may be a reservoir of *Arcobacter* spp. Over the last few years the presence of these organisms in raw meat products as well as in surface and ground water has received increasing attention.

The aim of this work is to assess the situation in Switzerland concerning the prevalence of *A. butzleri* in poultry, pig and cattle production line and to further characterize isolated strains.

A total of 1792 poultry samples, consisting of fecal swabs, neck skin, skin and skinless meat were taken. For cattle and pig, 568 and 602 samples, respectively, were examined, consisting of feces from the caecum, carcass surface swabs and retail meat samples.

The samples were enriched in CAT broth for 24 hours at 37°C, followed by an *A. butzleri* specific PCR. In order to isolate colonies, positive tested samples were cultured on selective media.

For poultry we found a prevalence of 1.4% (15/1090) for the fecal swabs, 53.6% (133/248) for the neck skin, 64.7% (134/207) for the skin and 15.1 % (36/238) for the meat. For cattle and pig, the prevalence for the feces was 0% (0/210) and 21.6% (54/250), for the surface of carcasses 19.7% (59/300) and 19.7% (59/300), and for retail meat 0 % (0/150) and 0% (0/52), respectively.

Isolated strains were further characterized by resistance testing, testing for adhesion capacity, cytolethal toxine detection and genotyping.

2 Introduction

Experts estimate that 76 million cases of human diseases, 325’000 hospital admissions and 5’000 mortalities are annually caused in the USA by the consumption of contaminated food (Mead et al. 1999). The importance of latent zoonoses has increased in recent years in view of food borne diseases: (i) the "healthy" animal represents a reservoir for specific pathogens; (ii) no pathological-anatomical changes in the carcass and its organs show the presence of these pathogens; and (iii) these pathogens may enter the food chain via weak points in the slaughtering or milking process.

Strict maintenance of good practices of slaughter hygiene in meat production is of central importance because microbiological hazards are not eliminated in the slaughtering process. Furthermore, to estimate the risks involved and to take appropriate measures, analysis of the slaughtering process should be complemented by collecting data related to the carriage of the animals of latent zoonotic pathogens.

There is evidence, that livestock animals may be a significant reservoir of *Arcobacter* spp., a potential latent zoonotic pathogen. Over the last few years the presence of these organisms in raw meat products as well as in surface and ground water has received increasing attention.

Arcobacter species are Gram-negative spiral-shaped organisms belonging to the family Campylobacteraceae that can grow microaerobically or aerobically. The arcobacter organisms also have the ability to grow at 25°C, which is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species. Recent evidence suggests that arcobacters, especially *A. butzleri*, may be involved in human enteric diseases. Moreover *A. butzleri* has also occasionally been found in cases of human extraintestinal diseases. However, up to now little is known about the mechanisms of pathogenicity or potential virulence factors of *Arcobacter* spp. Whilst the role of arcobacter in human diseases awaits further evaluation, a precautionary approach is advisable. In view of control measures to be used to prevent or to eliminate the hazard of *Arcobacter* spp. in food, several treatments have been evaluated for their effectiveness. Measures aimed at reduction or eradication of arcobacter from the human food chain should be encouraged.

But at present, with the available data from literature it is not possible to assess the significance of *Arcobacter* spp. as a human pathogen or as a food- and waterborne pathogen. Beside a standard isolation procedure for *Arcobacter* spp. detection, further studies would be needed to estimate the prevalence of *Arcobacter* spp. in patients with diarrhoea and to illuminate the pathogenic role and potential virulence factors of *Arcobacter* spp. Furthermore, additional information on the epidemiology of these microorganisms is also necessary. Up to now, no data is available for Switzerland. Therefore, the aim of this work is to assess the situation in Switzerland concerning the prevalence of *A. butzleri* in poultry, pig and cattle production line and to further characterize isolated strains.

3 Arcobacter, a potential food borne pathogen

3.1 Microbiology and molecular features

The family Campylobacteraceae includes the genera *Arcobacter* and *Campylobacter*, characterized as fastidious Gram-negative, non-spore-forming, motile, spiral-shaped organisms (Vandamme and De Ley, 1991). Together with the genus *Helicobacter*, they form a phylogenetically distinct group referred to either as rRNA super family VI or as the epsilon division of the class Proteobacteria (Vandamme et al., 1991).

To date, four classical species have been differentiated within the genus *Arcobacter*: *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. nitrofigilis* (Vandamme et al., 1992). Wirsen et al. (2002) described a coastal marine sulfide-oxidizing autotrophic bacterium, which produced hydrophilic filamentous sulfur as a novel metabolic end product. Phylogenetic analysis placed the organism in the genus *Arcobacter*. The proposed provisional name for the coastal isolate is "*Candidatus* Arcobacter sulfidicus". One more possibly new species has been identified by On et al. (2003) from pig abortions and turkey feces. Recently, Houf et al. (2005) isolated the new species *Arcobacter cibarius sp. nov.* from broiler carcasses. Furthermore, the first obligate halophile Arcobacter (*Arcobacter halophilus sp. nov.)* was found in water collected from a hypersaline lagoon on Laysan Atoll in the northwestern Hawaiian Islands (Donachie et al., 2005).

Arcobacter can grow microaerobically and aerobically and additionally has the ability to grow at 15°C, which is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species. A summary of the most important phenotypic traits for species identification is given in table 1.

Analysis of the ribosomal gene sequences has proven to be a valuable tool in determination of phylogenetic relationships between prokaryotes. Phylogenetic analysis of the 16S rRNA gene sequences of *Arcobacter* spp. strains and strains from closely related genera within the epsilon proteobacteria using the distance matrix tool of the ARB (latin, "arbor"=tree) package (http://www.arb-home.de/) shows moderate to high (94.2 – 98.6%) sequence similarity among the five published *Arcobacter* species type strain sequences (Accession numbers: L14625; L14624; L14627, AJ607391; AF314538). On the other hand similarity to other members of this phylum, namely *Campylobacter* spp., *Helicobacter* spp., *Sulfospirillum* spp. and

Wolinella spp. is low (< 90%). The distance matrix was calculated based on the small subunit rRNA sequence dataset (date 12-08-04) (table 2).

3.2 Pathogenesis and virulence factors

Up to now, little is known about the mechanisms of pathogenicity or potential virulence factors of Arcobacter. In a study of Musmanno et al. (1997) 18 isolates of *A. butzleri* from river water samples were examined for putative virulence characteristics. Toxin profiles based on cytotonic, cytotoxic and cytolethal distending factors were determined after analysis of responses induced in Vero and CHO cells. Adhesivity and invasivity tests were also performed on HeLa and Intestine 407 cells. All but one strain induced cytotoxic effects on cells in culture. The cytotoxic negative strain caused elongation of CHO cells (a cytotonic-like effect). This strain was the only one, which adhered to cells in vitro. Invasiveness was not observed in any strain tested. Overall, the results of this study showed phenotypic heterogeneity within arcobacters isolated from environmental sources, and indicated that some strains could potentially be virulent.

Johnson and Murano (2002) used a PCR system to screen *Arcobacter* spp. isolates from poultry, cattle, water and human diarrhea for the presence of cytolethal distending toxin (CDT) genes. There were no CDT gene positive isolates in this study but toxicity to HeLa and INT407 cells was observed. These cells were subsequently analyzed for cell cycle arrest in the presence of the arcobacter extracts with flow cytometry. Cells treated with arcobacter sonic extracts and filtrates exhibited normal cycle progression, confirming that CDT was not expressed by *Arcobacter* spp. Thus, *Arcobacter* spp. were shown to produce an entity that was toxic to some cells in culture, but its mechanism of action seemed different from that of *Campylobacter* CDT.

Recently, Villarruel-Lopez et al. (2003) investigated the cytotoxic effects of arcobacter strains isolated from retail meats. Ninety-five of the isolates were shown to induce some effect on Vero cells. A total of 38 isolates induced cell elongation, indicating enterotoxin production and 18 isolates induced vacuole formation. Meanwhile 39 isolates led to both vacuolization and elongation in Vero cells.

3.3 Potential role in human and animal diseases

In humans, *A. butzleri* and *A. cryaerophilus* have been isolated from stool samples of patients with acute diarrhea (Kiehlbauch et al., 1991; Burnens et al., 1992; Lerner et al., 1994; Engberg et al., 2000). Recently, Wybo et al. (2004) reported the first isolation of *A. skirrowii* from a patient with chronic diarrhea. However, the significance of *Arcobacter* spp. as a cause for human diarrhea is still unknown. This is probably due to the fact, that clinical samples are not routinely tested for *Arcobacter* spp. as is routinely done for *Salmonella* spp. or *Campylobacter* spp.

Arcobacters have, however, rarely been implicated in extraintestinal invasive diseases. Yan et al. (2000) reported *Arcobacter* spp. isolation from two blood cultures of a 60-year-old man with liver cirrhosis who presented with high fever and esophageal variceal bleeding. Lau et al. (2002) reported the isolation of *A. butzleri* from blood culture of a 69-year-old woman with acute gangrenous appendicitis.

Apart from *A. nitrofigilis*, the other species of *Acrobacter* spp. have also been isolated from various animal diseases including abortion, septicaemia, mastitis, gastritis and enteritis (Kiehlbauch et al., 1991; Anderson et al., 1993; Skirrow, 1994; Schroeder-Tucker et al., 1996; On et al., 2002).

3.4 Livestock as a reservoir for Arcobacter

Different recent studies reported *Arcobacter* spp. occurrence in feces of livestock animals detected by different isolation methods and molecular techniques (Wesley et al., 2000; Hume et al., 2001; Golla et al., 2002; Kabeya et al., 2003; Van Driessche et al., 2003; Öngör et al., 2004). Poultry in particular might be a significant reservoir of *Arcobacter* spp. However, there are conflicting reports in the literature, whether or not arcobacters are part of poultry intestinal microflora (Atabay et al. 1998; Eifert et al. 2003).

Moreover, *Arcobacter* spp. have been isolated from the intestine of healthy dairy cattle, pigs, sheep and horses. Van Driessche et al. (2003) validated a protocol previously developed for the *Arcobacter* spp. isolation from poultry for the isolation of *Arcobacter* spp. from feces of other livestock animals. Applying this method on fecal samples collected at slaughterhouses in Belgium, *Arcobacter* spp. was isolated from 44% of porcine, 40% of bovine, 16% of ovine and 15% of equine samples. All three animal-associated Arcobacter species were isolated and levels of up to 10^3 cfu

g^{-1} feces were found. In a recent study by the same group *Arcobacter* spp. were isolated from feces of healthy cattle on three unrelated Belgian farms, using a quantitative isolation protocol (Van Driessche et al. 2005). The prevalence on the three farms ranged from 7.5 to 15%. Of 276 animals examined, eight had a bacterial load of more than 10^2 cfu/g feces and low levels were detected in 22 animals using enrichment. *Arcobacter cryaerophilus* was the dominant species isolated from cows. Öngör et al. (2004) detected *Arcobacter* spp. in 9.5% of 200 rectal fecal samples collected from cattle in Turkey.

Another survey was done by Kabeya et al. (2003) examining the distribution of *Arcobacter* spp. among livestock in Japan. *Arcobacter* spp. were isolated from 3.6 and 10% of the cattle and swine fecal samples, respectively, along with 14.5% of chicken cloacal swabs. *A. butzleri* was the most prevalent species.

3.5 Occurrence in foods, source and mode of transmission

Because there is no standard isolation method for *Arcobacter* spp. detection, the true occurrence of this potential pathogen in food is largely unknown. In addition, the lack of a standardized isolation protocol limits the ability to compare field data.

It has been suggested that water may play an important role in the transmission of these organisms and drinking water has been cited as a major risk factor in acquiring diarrheal illness associated with Arcobacter. Different studies reported the detection of *Arcobacter* spp. in surface water, ground water, sewage and activated sludge (Musmanno et al., 1997; Jacob et al., 1998; Rice et al., 1999; Stampi et al., 1999; Fera et al., 2004; Morita et al., 2004). Moreover, Assanta et al. (2002) showed in their study, that arcobacter cells could easily attach to various water distribution pipe surfaces, such as stainless steel, copper and plastic. Extracellular fibrils were observed on the stainless steel surface, especially after 72 h.

Raw meat is considered as another source of arcobacter infection in humans (table 3). However, little is known about the epidemiology of *Arcobacter* species in slaughterhouses. Investigation revealed that *A. butzleri* and *A. cryaerophilus* are commonly present on slaughter equipment (Houf et al., 2002). Gude et al. (2005) examined the ecology of *Arcobacter* species in chicken rearing and processing. They did not find *Arcobacter* spp. in samples from the live birds and their immediate environment, but *A. butzleri* was found in effluent sludge and stagnant water outside

the rearing sheds. Furthermore they found that *A. butzleri* was very widely distributed throughout the abattoir environment. Therefore it seems likely that poultry carcasses are mainly contaminated during processing. However, contamination of poultry carcasses through slaughter equipment alone could not explain the high contamination levels (log 2 –log 3/g neck skin) that were found in a study on Belgian poultry products (Houf et al., 2002). The carcass contamination must therefore additionally have another unknown source.

3.6 Growth, survival and inactivation

Generally, *Arcobacter* spp. grow microaerobically and aerobically. For a selected *A. butzleri* strain a temperature range from 15 °C to 37 °C with a specific growth rate at 30 °C was reported (Hilton et al., 2001). In agreement with a study done by Vandamme et al. (1992), there was no detectable growth at 40 °C. The ability to grow at 15 °C ($_{td}$ 17 h) is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species (Mansfield and Forsythe, 2000; Hilton et al., 2001).

Moreover, this *A. butzleri* strain grew between pH 5.0 and 8.5, with optimal growth observed between pH 6.0 and 8.0 at 30 °C. Meanwhile at 37 °C its minimum pH limit increased to pH 5.5 (Hilton et al., 2001). In the same study the effect of cold storage on *A. butzleri* was studied at chilled (4 °C) and freezer (-20°C) temperatures. Storage of cells from the stationary phase at 4 °C caused a gradual decrease (log 4) over 21 days. However, freezing caused a log 2 decrease in viability after only 24 h storage and thereafter the viability remained constant.

In view of control measures to be used to prevent or to eliminate the hazard of *Arcobacter* spp. in food, several treatments have been evaluated for their effectiveness in eliminating *Arcobacter* spp. Hilton et al. (2001) examined the thermo tolerance of the organism in broth cultures. Decimal reduction times for *A. butzleri* at 55 °C were 0.4 min for cells in the stationary growth phase and 1.1 min for those in the exponential growth rate. These data are comparable with the D-values published for *C. jejuni* (55°C, 0.64-2.13 min) (Anonymous, 1996). The z-values for exponential and stationary phase harvested cells were 8.1 °C and 7.4°C, respectively.

The effect of preservatives has also been studied. At 0.5%, 1.0% and 2.0% lactic and citric acids inhibited *A. butzleri* growth, 2% sodium lactate was effective in inhibiting growth over 8 h incubation but not over longer periods (Phillips, 1999). Nisin alone

inhibited *A. butzleri* growth over 5 h. It did not enhance the effect of sodium citrate inhibition but it did augment the effect of sodium lactate over 8 h (Phillips, 1999). Short-term treatment with both trisodium phosphate and EDTA, alone or in combination with nisin are effective in reducing survival of *A. butzleri* in pure cultures (Phillips and Duggan, 2001).

3.7 Detection, identification speciation and typing methods

Cultural detection of *Arcobacter* is generally performed by an enrichment step under aerobic conditions at 25 °C and takes on average four to five days until the identification of a suspected *Arcobacter* spp. colony is completed. Commercially available isolation media include cefoperazone, amphotericin B and teicoplanin agar (CAT) for *Arcobacter* spp. and charcoal cefoperazone, deoxycholate agar (CCDA) that is more specific for *A. butzleri* (www.oxoid.com). A comprehensive review of isolation and identification methods was published recently by Corry et al. (2003).

In the last few years several studies comparing different protocols have been published. Johnson and Murano (1999) compared three different protocols for the isolation of *Arcobacter* from poultry. In this study they found the best results by combining JM enrichment broth with JM agar. In another study the sensitivity of three methods used in the isolation of *Arcobacter* spp. from spiked raw ground pork was tested (Ohlendorf and Murano, 2002). The JM method was determined to be the most sensitive, detecting *A. butzleri* down to a level of 10 CFU/g in 100% of the samples and detecting *A. cryaerophilus* at 10 CFU/g in 75% of the samples. In a pure buffer system, the Collins method was as effective as the JM method in isolating both organisms to a level of 10 cfu per g. Recently, Scullion et al. (2004) compared three methods for the isolation of *Arcobacter* spp. from retail raw poultry. Method 1 (On et al., 2002) was microaerobic and involved a membrane filtration step followed by plating onto blood agar. Method 2 (Houf et al., 2000) was also microaerobic and involved enrichment and plating media containing a five-antibiotic cocktail. Method 3 (Johnson and Murano, 1999) was aerobic and was based on enrichment in a charcoal-based broth containing two antibiotics. In terms of sensitivity, ease of use and diversity of species recovered the authors propose method 3 by plating the enrichment broth at 48 h as the method of choice.

Over the last years DNA-based assays were also established for rapid and specific identification of *Arcobacter* spp. (Harmon and Wesley, 1996; Hurtado and Owen, 1997; Marshall et al., 1999; Al Rashid et al., 2000; Gonzalez et al., 2000; Winters and Slavik, 2000; Moreno et al., 2003). Moreover multiplex PCR systems targeting the 16S and 23S rRNA genes have been described for the simultaneous detection and identification of different *Arcobacter* spp. (Harmon et al., 1997; Houf et al., 2000; Fera et al., 2004). Furthermore, Atabay et al. (1998, 2003) used SDS-PAGE method of whole-cell proteins for species level identification of *A. butzleri*.

Determining the relatedness of isolates has become increasingly important in epidemiological studies. Phenotyping methods such as biotyping or antibiograms are of limited use in discrimination of strains to the subspecies level. The development and application of molecular techniques has provided alternatives for typing of strains. The PCR based approaches that have been applied for *Arcobacter* spp. strain discrimination include the random amplification of polymorphic DNA (RAPD) and an enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) (Atabay et al., 2002; Houf et al., 2002).

Atabay et al. (2002) used RAPD-PCR technique for subtyping 35 *A. butzerli* isolates from chicken carcasses and eleven distinct DNA profiles were obtained. RAPD-PCR technique was found to be a useful technique to reveal epidemiologic association among strains. Houf et al. (2002) optimized in their study an ERIC-PCR and a RAPD-PCR for characterization of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. In addition, a simple and rapid DNA extraction method was tested for use in both typing procedures. In their study both methods had satisfactory typeability and discriminatory power, but the fingerprints generated with ERIC-PCR were more reproducible and complex than those obtained with RAPD-PCR. Both methods were further applied for molecular characterization of *Arcobacter* spp. isolates collected in a poultry slaughterhouse in Belgium (Houf et al., 2003). Characterization of 1079 isolates resulted in the delineation of 159 *A. butzleri* and 139 *A. cryaerophilus* types. With these results the routes of transmission could not be clarified. However, the data brought to light insufficient cleaning and disinfection at the processing plant and confirmed the survival capacity of certain *A. butzleri* strains.

While PCR based methods seem to be advantageous in time saving, pulsed-field gel electrophoresis (PFGE) is generally considered to be the “gold standard” for most of the relevant pathogens in food hygiene. Hume et al. (2001) used PFGE for

genotyping *Arcobacter* spp. from nursing sows and growing pigs on three farms. They found little similarity between genotypic patterns for isolates from the three farms. The level of genotypic variation suggested that pigs in this furrow-to-finish operation were colonized by multiple Arcobacter parent genotypes that may have undergone genomic rearrangement, common to members of Campylobacteraceae, during successive passages through the animals. Furthermore, Arcobacter strains isolated from ground chicken, pork, beef and lamb meats were genotyped by PFGE (Rivas et al., 2004). A number of isolates with indistinguishable fingerprints were found. Results from these two studies show, that PFGE can also be used as a genotyping tool for Arcobacter to investigate epidemiological questions or during outbreaks.

3.8 Risk management strategies

A definitive link between Arcobacter and human diseases has not been established yet, but public health concerns have been raised due to the fact that there are several opportunities that exist for human exposure to arcobacters. Currently, a major focus is on raw meat products. Furthermore unpasteurized dairy products may also pose a risk of arcobacter transmission to the human population. Therefore, whilst the role of arcobacter in human disease awaits further evaluation, a precautionary approach is advisable. Measures aimed at reduction or eradication of arcobacter from the human food chain should be encouraged.

The fecal carriage of food borne pathogens among livestock animals at slaughter is strongly correlated with the hazard of carcasses contamination at the slaughter line (Bonardi et al., 2001; Beach et al., 2002). In order to reduce the risk represented by zoonotic agents to the consumer health, it is essential to reduce contamination of carcasses during the slaughtering processes. Therefore, the maintenance of slaughter hygiene is consequently of central importance in meat production. It can be measured in daily practice by “in-process-controls” and regular microbiological monitoring of carcasses as a verification system according to the HACCP principles. Moreover, preventive measures, such as implementation of codes of good manufacturing practices, increased care during hiding and evisceration should be encouraged.

4. Materials and Methods

This study was based on sampling carried out within nine months (from October 2004 to June 2005).

4.1 Samples

4.1.1 Chicken samples

The chicken samples originated from one of the two big slaughterhouses for poultry in Switzerland. Samples were taken on Monday, Tuesday and Wednesday, subsequently placed into a cool box and sent over night to the laboratory. Microbiological examinations were carried out the next day.

Samples consisted of 1099 fecal swabs taken from the crates, 248 neck skins after plucking, as well as 207 skins and 238 skinless chicken breasts along the slaughter lines. At each sampling the 30 fecal swabs were taken straight after arrival at the slaughterhouse from three different flocks. The neck skin samples were also collected from these three flocks. The skin and chicken breast samples were collected three times throughout a working day as pool samples of ten workplaces.

4.1.2 Cattle and pig samples

The fecal samples and the samples of carcasses of cattle and pigs were collected in a EU-approved slaughterhouse, where cattle and pigs were slaughtered on separate mechanized lines. Sampling took place on Monday, Tuesday and Wednesday. An average of about 15 samples was taken on every single day. These samples were taken from as many different herds as possible. They were transported chilled to the laboratory.

460 fecal samples were collected from the caecum of just-slaughtered animals and placed in sterile plastic tubes.

508 samples originated from the surface of slaughtered animals stored in the cooling room. They were obtained by the wet-dry double swab technique from four different sites (4x 100cm^2) stipulated by the EU Decision (cattle: neck, brisket, flank and rump; pig: back, neck, ham and belly). At each sampling site, a moistened swab (0.1% buffered peptone water + 0.85% sodium chloride solution) was rubbed vertically,

horizontally and diagonally across the sampling site delineated by a template. Then the same sampling procedure was repeated by a dry swab.

Furthermore, 150 samples of minced beef meat and 52 samples from pork meat were taken at retail level. Microbiological examinations were carried out about 2 h after sampling.

4.2 Arcobacter detection

4.2.1 Enrichment step

5 g skin or meat was transferred into a sterile stomacher bag. Next, 45 ml sterile Arcobacter enrichment broth CAT (CM 965, Oxoid Ltd., Hampshire, UK) with supplement (SR 174, Oxoid, Ltd., Hampshire, UK) was added. The mixtures were homogenized for 30 s with a stomacher blender at normal speed. After homogenization they were incubated for 24 h under aerobic conditions at 37 °C. The fecal swabs were put into sterile glass tubes filled with nine ml CAT broth and incubated as described above.

The surface swabs were also transferred into a sterile bag and CAT broth was added until the swabs were totally covered. After homogenization with the blender they were enriched the same way as the other samples.

4.2.2 PCR Screening for the presence of *A. butzleri*

The enrichments were screened by PCR for the presence of *A. butzleri.* All PCR assays applied in this study were performed in a T3 thermocycler (Biometra, Göttingen, D). PCR reagents were purchased from PROMEGA (Madison, Wisconsin, USA), and primers were synthesized by MICROSYNTH (Balgach, CH). The 50 µl PCR mixtures consisted of 2 µl of enrichment suspension boiled at 100 °C for 11 min in 42 µl of double-distilled water, 5 µl of 10-fold-concentrated polymerase synthesis buffer containing 2.0 mM $MgCl_2$, 200 µM (each) desoxynucleosid triphosphate (dNTP), 30 pmol of each primer (primer ARCO 5'-CGTATTCACCGTAGCATAGC-3' and primer BUTZ 5'-CCTGGACTTGACATAGTAAGAATGA-3' (target 16S rDNA, Houf et al., 2000) per sample, and 2.5 U of *Taq* DNA polymerase.

PCR involved 32 cycles of denaturation (94 °C, 45 s), primer annealing (61 °C, 45 s) and chain extension (72 °C, 30 s).

PCR products were analyzed by electrophoresis at 76 V for 30 min through a 1,8% standard agarose (Eurobio) gel. The amplimers (401-bp fragments) were visualized by staining with ethidium bromide under UV light. A 100-bp DNA ladder was used as molecular weight marker.
For confirmation, sequencing of some amplification products purified with a Min Elute Gel Extraction Kit (Qiagen) was determined by the dideoxynucleotide triphosphate chain termination method of Sanger, with the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3100 Genetic Analyzer (Applied Bio-Systems).

4.2.3 Subculturing and strain identification

PCR positive samples were streaken on CSA plates (Difco 0964-17-5, Becton Dickinson, Sparks, USA) with lyzated horse blood (SR48, Oxoid Ltd., Hampshire, UK) and Butzler Campylobacter selective supplement (SR85, Oxoid Ltd., Hampshire, UK) and incubacted under microaerobic conditions for 48 h at 25 °C. Suspect colonies (small, creamy-grey to transparent) were picked from the plates, subcultured onto CSA plates and incubated for another 48 h under the same conditions. For identification of the subcultered colonies GRAM staining (small gram-negative curved rods), as well as biochemical tests such as oxidase and catalase tests (both positive) were made and finally confirmed by a second PCR (See 4.2.2).

4.2.4 Further strain characterization

4.2.4.1 Resistance testing

The E-test (AB Biodisk, Solna, Sweden) was performed for erythromycin, ciprofloxacin and tetracycline on Mueller-Hinton agar supplemented with 5% sheep blood according to the instructions provided by the manufacturer. Inocula were prepared by incubating the strains for 48 h at 37 °C under aerobic conditions in trypticase soy broth. After application of the E-test strips, plates were incubated in in microaerophilic conditions at 37 °C for 48 h. The minimal inhibition concentrations (MIC) were read directly from the test strip at the point where the elliptical zone of inhibition intersected the MIC scale on the strip. The following NCCLS MIC breakpoints for resistance were applied: ciprofloxacin MIC ≥ 4 mg/l, erythromycin MIC ≥ 8 mg/l, tetracycline MIC ≥ 16 mg/l.

4.2.4.2 PCR amplification of cytolethal distending toxin genes

The isolated *A. butzleri* strains were grown in BHI broth for 48 h at 37 °C. DNA was extracted from the enrichments by using the DNeasy Tissue Kit (Qiagen, AG, CH) in accordance with the suppliers' protocol. As a positive control *C. jejuni* was used. The *cdtB* genes were amplified by PCR's with the degenerative forward oligonucleotide primer MIX5' with the nucleotide sequence 5'-GAA ARY AAA TGG ARY RYW MRT GTM MG-3', and the reverse oligonucleotide primer MIX3' 5'-AAA TCW CCW RSA ATC ATC CAG TTA-3' as previously described (Yamano et al. 2003). 2 µl of DNA was mixed with 5 µl 10xPCR buffer, 2 µl $MgCl_2$, 1 µl dNTP, 0.5 µl of each oligonucleotide primer, and 0.4 µl of FastStart Taq DNA polymerase (Roche). The PCR parameters for amplification reactions were: 5 min 94 °C, 40 cycles of 94 °C for 30 s, 42 °C for 2 min 72 °C for 30 s, and 72 °C for 5 min. PCR products were analyzed by electrophoresis at 76 V for 30 min through a 1.8% standard agarose (Eurobio) gel. The amplimers were visualized by staining with ethidium bromide under UV light. A 100-bp DNA ladder was used as molecular weight marker.

4.2.4.3 Adhesion assay

A. butzleri strains were characterized by the adherence capacity to HEp-2 cells as described by Karch et al. (1993) with minor modifications. Briefly, HEp-2 cells were grown for 48 h with 5% CO_2 on sterilized coverslips (13 mm diameter; Bibby Sterilin™ Ltd., Stone, UK) in 24-well flat-bottom tissue culture plates with low evaporation lid (16 mm diameter; BD Falcon™, Bedford, USA) containing Minimum essential medium (MEM with Earle`s salts, 25 mM HEPES, without L-Glutamine;GIBCO™ Invitrogen AG, Basel, CH) supplemented with fetal calf serum (FCS, 10%, Bio Concept™, Allschwil, CH), MEM Non Essential Amino Acids (MEM NEAA (100x) without L-Glutamine, GIBCO™ Invitrogen AG, Basel, CH) and GlutaMAX™I supplement 200mM (100x), (GIBCO™ Invitrogen AG, Basel, CH). Bacteria used for the adhesion assay were grown over night in BHI-broth. Prior to incubation, the confluent cells were washed with Dulbecco`s phospate buffered saline (D-PBS (1x) without Calcium and Magnesium, GIBCO™ Invitrogen AG, Basel, CH). 200 µl of the *A. butzleri* enrichment broth and 1 ml fresh media (MEM with supplements) was added to the cells and was incubated for 3 h at 37 °C and 5% CO_2. After this incubation period, monolayers were washed six times with D-PBS and the cells were fixed for 10 min with 4Methanol. After the staining with May-Grünwald-

Giemsa (Fluka™ , Buchs SG, CH), the coverslips were examined by light microscopy to determine the adherence patterns of the strains. *E. sakazakii* strain ES 5 was used as a positive control.

4.2.4.4 Genotyping by ERIC-PCR

Genotyping of the isolated strains was performed by an ERIC-PCR. The strains were grown in BHI broth for 48 h at 37 °C. DNA was extracted from the grown colonies using the DNeasy® Tissue Kit (Qiagen AG, CH) in accordance with the suppliers' protocol. 10 µl of DNA template was used for each experiment.

For ERIC-PCR, reaction mixtures (total volume 25 ml) containing primers ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') (Atabay et al., 2002) at 10 pmol each were prepared by using 10 x *Taq* reaction mixture, 2 U *Taq* polymerase (Promega, Madison, WI),) and 200 mM dNTPs each.

The amplification was performed in a T3 thermocycler (Biometra, D). The PCR conditions were: 5 min at 95 °C, 39x (95 °C, 1 min; 25 °C, 1 min; 72 °C, 4 min). Cycling was completed by a final elongation step at 72 °C for 8 min. After PCR the reaction products were separated on a 1.5% agarose gel, stained with ethidium bromide and visualized under UV light.

4.3 Campylobacter detection

4.3.1 Enrichment step

Poultry samples were additionally tested for Campylobacter. 5 g skin or meat was transferred into a sterile stomacher bag. Next, 45 ml sterile Campylobacter selective broth CSB (Difco 0495-17-3, Becton Dickinson, Sparks, USA) with supplement (SR84, Basingstoke, Hampshire, UK) was added. The mixture was homogenized for 30 s with a stomacher blender at normal speed. After homogenization 9 ml of the suspension was transferred into a sterile glass tube. The fecal swabs were also put into sterile glass tubes filled with 9 ml CSB. The tubes were all transferred into a jar and enriched for 24 h at 43 °C under microaerobic conditions provided by commercial gas packs (CampyPak Plus, BBL 271045, Becton Dickinson, Cockeyville, USA).

4.3.2 Subculturing and strain identification

After incubation the enrichments were streaked on CSA plates and incubated for 48 h at 43 °C under microaerobic conditions in a jar. Following incubation, the plates were checked for bacterial growth. Suspect colonies were taken for further identification. Identification was carried out using the following standard tests: GRAM staining (small gram-negative curved rods), oxidase and catalase reactions (both positive). In order to distinguish *C. jejuni* from *C. coli,* grown bacterial colonies were transferred into a tube for hippurat reaction and incubated for 24 h at 37°C under aerobic conditions. The next day two drops of Ninhydrine (Bio Mérieux SA, Marcy l'Etoile, F) were added and 15 min later the tubes were checked for purple coloring, which indicates the presence of *C. jejuni*.

5 Results

5.1 Arcobacter detection method

5.1.1 PCR screening for the presence of *A. butzleri*

As an example the typical PCR run products from various enriched samples with a positive control are shown in figure 1.

For confirmation sequencing of some amplification products was made. Figure 2 shows an alignment of *A. butzleri* reference strain (ATCC 49616) 330 bp region (nt. 977 – 1303) against the sequence of PCR products amplified from the corresponding region of *A. butzleri* isolates. There is a 100% homology to the reference strain of *A. butzleri*.

5.1.2 Arcobacter culture

In preliminary examinations the enriched samples were streaked on CAT Agar (CM 739, Oxoid Ltd., Hampshire, UK) with Campylobacter selective supplement (SR174, Oxoid Ltd., Hampshire, UK) and the plates were incubated for 24 h at 37 °C. There was no growth of the expected small colonies; therefore the plates were incubated for another 24 h. Then the plates were mostly covered with competing microflora, so that it was impossible to detect the suspect colonies. This was also described by Atabay et al. (1998).

In order to improve the cultural detection method, different temperature, time, aerobic and microaerophilic conditions were examined. As a control, there was always a reference strain carried along with every method.

We found, that the best way of reducing the competing microflora, which consisted mainly of *Pseudomonas* spp., was to plate the samples on CSA Agar and incubate them in a jar for 48 h at 25 °C under microaerobic conditions. With this procedure the reference strain was also growing without any problems.

5.2 Prevalence data

The *A. butzleri* prevalence data in the various samples are comparatively shown in table 4.

5.2.1 Arcobacter and Campylobacter prevalence data for poultry

5.2.1.1 Arcobacter prevalence data for poultry

Of the fecal samples obtained from the crates 1.4% (15/1090) were found to be PCR positive. 53.6% (133/248) of the neck skin samples were tested PCR positive. For the skin samples, taken from further processing stages of carcasses, 64.7% (134/207) were detected PCR positive. Furthermore, 15.1% (36/238) of the meat samples were positive for *A. butzleri*.

5.2.1.2 Campylobacter prevalence data for poultry

Only poultry samples were tested for Campylobacter. The prevalence data for *C. jejuni* and *C. coli* are shown in table 5. 164 (14.9%) *C. jejuni* and five (0.5%) *C. coli* strains were isolated from 1099 fecal samples taken from the transport cages. All *C. coli* isolates originated from the same flock. For neck skin, skin and meat only *C. jejuni* was detected. 51 (20.9%) out of 244 neck skin samples, 14 (6.9%) out of 204 skin samples and 17 (7.7%) out of 220 meat samples were found positive for *C. jejuni*.

In 40 (16,4%) out of 244 flocks, the fecal samples as well as the neck skin samples were positive for *C. jejuni*, whereas in 21 (8.6%) out of 244 flocks *C. jejuni* was only found in the feces and in 11 (4.5%) out of 244 flocks only the neck skin was detected positive for *C. jejuni*. 172 (70.5%) flocks were tested Campylobacter negative.

5.2.1.3 Seasonality of Arcobacter and Campylobacter prevalence on poultry flock level

No seasonality of Arcobacter prevalence was found in poultry flocks (table 6). In comparison to that, there is tendency to seasonality of occurrence of Campylobacter in fecal samples and significant seasonality (chi^2 test, $p<0,05$) in neck skin samples with an increase in the prevalence of Campylobacter in springtime.

5.2.2 Arcobacter prevalence data for cattle

None (0/210) of the fecal samples from caecum were detected PCR positive. Of the carcass samples obtained by the wet-dry double swap technique 19.7% (41/208) were detected positive. There was no meat sample (0/150) detected positive.

5.2.3 Arcobacter prevalence data for pig

Of the fecal samples from caecum 21.6% (54/250) were PCR positive. 19.7% (59/300) of the carcasse surface samples obtained by the wet-dry double swap technique were tested positive. Among the meat samples no (0/52) *A. butzleri* was detected.

On a single day, the prevalence of *A. butzleri* in the skin samples was 68.2% (15/22). That day, more than 1000 pigs slaughtered, before the samples were taken.

5.3 Further strain characterization

In total 22 *A. butzleri* strains, 7 from poultry, 6 from cattle and 9 from pig were taken for further strain characterization. 6 poultry strains originated from neck skin and one strain from carcasses. For the cattle all strains were isolated from carcasses. 8 pig strains originated from carcasses and one from feces.

5.3.1 Resistance testing

The results of susceptibility testing using the E-test are shown in table 7. The E-test MICs for ciprofloxacin ranged from 0.064 to 0.25mg/l, those for erythromycin ranged from 0,19 to 6,0 mg/l and those for tetracycline ranged from 0.125 to 3.0 mg/l. No resistances to the three tested antibiotics were found.

5.3.2 PCR amplification of cytolethal distending toxin *cdt*B gene

It was investigated whether the degenerative oligonucleotide primers MIX5' and MIX3' designed, based on various regions of the *E. coli* and Campylobacter *cdt*B genes, which have been successfully used for the identification and cloning of *ctd*ABC genes from various bacteria, would produce an appropriate PCR product when DNA from the 22 *A. butzleri* strains was used as the template. Only a single product of the expected size (ca. 470 bp) was observed for the Campylobacter strain used as positive control (figure 3).

5.3.3 Adhesion assay

Three isolates out of 22 showed adhesion to cells. The adhesion of one isolate is illustrated in figure 6. All three isolates originated from poultry neck skin samples and they adhered with different intensity (see table 9).

5.3.4 Genotyping by ERIC-PCR

The 22 *A. butzleri* strains isolated from chicken, cattle and pig were further genotyped by an ERIC-PCR. From these isolates nine different DNA patterns were obtained. These patterns are shown in figure 4 and figure 5. The distribution of the different patterns was: two of the seven chicken strains belong to pattern II, two of them to pattern III, and one each to pattern V, VI and VII. Of the nine pig strains five show pattern I and four of them pattern IV. Five of the six strains of cattle belong to pattern VIII and one of them to pattern IX (table 8). There was never found the same genotype of *A. butzleri* within different species.

6 Discussion

Arcobacter spp. have become more important over the last few years as a potential food related pathogen. They have been isolated from animals, chicken carcasses and humans with diseases (Phillips, 2001), as well as from drinking water treatment plants (Jacob et al., 1998; Rice et al., 1998). *A. butzleri* is the most commonly reported human pathogen of the *Arcobacter* species associated with human diseases, such as enteritis, severe diarrhea with abdominal pain, vomiting, fever and chills (Kiehlbauch et al., 1991; Vandamme et al., 1992; Engberg et al., 2002). For example, *A. butzleri* was isolated from fecal samples in an outbreak of recurrent abdominal cramps at a nursery and primary school in Italy (Vandamme et al., 1992). DNA fingerprints of these outbreak-related strains of *A. butzleri* were found to be identical (Vandamme et al., 1993). Adding to this, Arcobacters appear to be resistant to antimicrobial agents typically used in the treatment of diarrhea illness caused by *Campylobacter* spp., e.g. erythromycin, other macrolide antibiotics, tetracycline and chloramphenicol. They are also resistant to clindamycin (Kiehlbauch et al., 1992). This, however, is contrary to our results and to results of other studies. In our study, resistance to ciprofloxacin, erythromycin and tetracycline was tested and we found no resistances for our strains from poultry, cattle and pig samples. Several other authors have discussed this subject. Houf et al. (2004) determined the MICs of five antimicrobial agents by the agar dilution method for 98 *A. butzleri* and 28 *A. cryaerophilus* strains from humans and poultry. With ciprofloxacin, a bimodal distribution of susceptibility levels was seen for human *A. butzleri* isolates (0.015-0.03 versus 0.12-0.25), whereas MICs for 65 of the 68 *A. butzleri* poultry strains ranged from 0.12 to 0.5 mg/ml and only three strains from three different broilers were resistant with a MIC of 16 mg/ml. One *A. cryaerophilus* strain from poultry was resistant to erythromycin at a MIC of 128 mg/ml, whereas MICs for the other Arcobacter strains ranged from 2 to 32 mg/ml. Fera et al. (2003) examined 17 strains of *A. butzleri* and 13 of *A. cryaerophilus* for their antimicrobial susceptibility to 26 antimicrobial agents. They also described good susceptibility to ciprofloxacin. Another study carried out by Kabeya et al. (2004) found no resistance to tetracycline and erythromycin among the 122 *Arcobacter* spp. strains tested. Atabay and Aydin (2001) tested 39 *A. butzleri* strains isolated from broiler chickens for their

susceptibility to 23 antimicrobial agents using a disc diffusion method. One isolate was resistant to, and four showed intermediate level of resistance to erythromycin. All isolates were susceptible to enrofloxacin and tetracycline.

Nevertheless, the significance of *A. butzleri* as a human pathogen is, as yet, not fully evaluated. This might be due to the facts, that on the one hand patients suffering from diarrhea and abdominal pain are rarely tested for Arcobacter and on the other hand, there is no standard screening procedure to detect Arcobacter so far. In our study, we succeeded in isolating 22 *A. butzleri* strains even though we faced the problem of culturing *A. butzleri* during our work. This was mainly due to the competing microflora, which was often seen to overgrow the plates. In order to inhibit this competing microflora we tried to improve the cultural detection method and finally found a procedure to reduce the competing microflora, which is described in Materials and Methods. Whilst the role of arcobacter in human disease awaits further evaluation, a precautionary approach is advisable. This means that it is also nessessary to accomplish more data for risk assessment. Although there is evidence that livestock animals may be a significant reservoir of *Arcobacter* spp., no data at all was available for Switzerland up to now.

In poultry the low prevalence (1.4%; 15/1090) for *A. butzleri* in feces found in our study is comparable with previous findings (Atabay and Corry, 1997; Corry and Atabay, 2001; Houf et al., 2002), that arcobacters do not colonize the poultry intestinal tract, and with a study of Van Driessche et al. (2003) who isolated arcobacters from feces of other livestock but not from poultry feces. A recent study from the UK confirms these results by finding no *Arcobacter* spp. in all the fecal samples examined (Gude et al., 2005). These authors found, that *Arcobacter* spp. do not appear to be part of the normal flora of chickens during rearing and natural infections are rare. A study in Iowa, where they found 1% of 407 cloacal swaps positive for *A. butzleri* confirms this fact further (Wesley and Baetz, 1999). Kabeya et al. (2002) describe in Japan a prevalence of 6.8% of *A. butzleri* in fecal samples. The prevalence data of these studies are summarized in table 10. In all studies mentioned, positive samples were detected by culturing after enrichment. Suspect colonies were subcultered and finally Arcobacter was identified by genus-specific PCR.

The prevalence of Campylobacter in the investigated poultry fecal samples, 15.4% (169/1099), is higher than the prevalence found for *A. butzleri.* On flock level, 66 out of 254 (26.5%) flocks were tested positive for *Campylobacter* spp. Only one flock (1,5%) out of the 66 positive flocks was positive for *C. coli*, while 65 flocks (98.5%) were positive for *C. jejuni*, which corresponds with other findings. Berndtson et al. (1996b) found, that in 75 out of 77 positive flocks (97%) the isolated strains were *C. jejuni* and only in two flocks the isolated strains were *C. coli*. In most cases (92%), all five pooled fecal samples from a flock were either tested positive or negative for Campylobacter, which indicates, that once Campylobacter enters a flock, it spreads rapidly and widely and infects almost all birds. This has already been revealed by others (Smitherman et al., 1984; Berndtson et al., 1996b, Ring et al. 2005). In our study, the prevalence for *A. butzleri* on neck skin was 53.6% (133/248). Houf et al. (2001) describe a prevalence of 36% for *A. butzleri* (16/46) on neck skin after plucking. They detected the positive samples by direct isolation without an enrichment step and identified the isolated strains by a m-PCR assay, whereas Gude et al. (2005) found two neck skin samples out of two positive by culturing after enrichment. In our study 134 out of 207 samples (64.7%) from carcasses at further stage of processing were tested positive for *A. butzleri.* Recently Atabay et al. (2003) also found a prevalence of 65 % by testing 75 chicken carcasses (44 fresh and 31 frozen) sold in retail markets in Turkey. He isolated the strains after enrichment and finally performed a m-PCR for identification. Three further studies, carried out in different countries (see table 11), confirm the high prevalence for *A. butzleri* on poultry carcasses.

The rather high prevalence for *A. butzleri* in our study in samples taken from carcasses at the slaughterhouse compared with the low prevalence found in feces, indicates that a contamination with Arcobacter in the abattoir is more likely than a direct fecal contamination. This has also been described in a study by Wesley and Baetz (1999), who found, that despite its distribution on poultry carcasses, *Arcobacter* spp. have only been infrequently recovered from the caecum. The occurrence of *A. butzleri* during slaughter process might be traced back to two possible ways of contamination of carcasses. Either, arcobacters may colonize the environment of a slaughterhouse and bring a constant level of contamination on carcasses or arcobacters may be carried into the slaughterhouse through an arcobacter positive flock in the morning and distributed on all following carcasses

through contamination of the plucker or other processing machines used. Gude et al. (2005) suggest, that Arcobacter appears to colonize the abattoir environment and chicken carcasses during processing, but that the numbers appear to be low. Houf et al. (2002) support this finding. They examined samples taken from slaughter equipment and processing machines before slaughter in the morning and after a 3-day period with no slaughter activity and revealed that *A. butzleri* was commonly present. The consistently high presence of *A. butzleri* on carcasses during processing indicates, that the contamination takes place at an early stage of processing and cannot be eliminated until the final product. Houf et al. (2002) found, that arcobacters persist in the abattoir even after cleaning and disinfection. So, the similarly high contamination levels throughout all stages of processing might be an indication for inadequate disinfection of the abattoir or for the capacity of Arcobacter to survive in an abattoir environment. Nevertheless, it appears unlikely, that the high prevalence of *A. butzleri* on carcasses during slaughter is only due to the contamination of slaughter equipment. By genotyping with ERIC-PCR we detected a high heterogeneity within poultry strains isolated from carcasses. We found five different patterns among seven strains of *A. butzleri*. Samples obtained on different days always carried a different genotype of *A. butzleri*. This suggests that there are numerous sources of contamination and that broad genotype diversity may exist within poultry flocks. Furthermore, these results indicate that *A. butzleri* is more likely to enter the slaughterhouse on the animals in the morning than being part of the environmental flora. After all, these findings raise some questions, which remain to be investigated in the future.

For Campylobacter, we found a prevalence of 20.9% (51/244) for the neck skin samples, which is close to the prevalence of 15.4 % we found for the fecal samples taken from the crates. Adding to this, 40 out of 72 flocks were detected campylobacter positive for feces and neck skin. This suggests that a primary contamination is likely to occur here. This corresponds with the finding of Jacobs-Reitsma (2000), that potential sources of Campylobacter contamination of poultry carcass include, among others, fecal contamination of feathers and skin during transportation to the slaughter facility. It is known, that stress can cause a disturbance of intestinal functions and may lower the resistance of the live animal and increase spreading of intestinal bacteria (Keener et al., 2004). In our study, the detected prevalence for *Campylobacter* spp. on carcasses at further stages of

processing is with 6.9% (14/204) lower than the prevalence for *A. butzleri* in the same samples (64.7%). Houf et al. (2002) also found a lower prevalence for *Camplyobacter* spp. than of Arcobacter on Belgian poultry carcasses. This finding may suggest, that the thermophilic Campylobacters are less resistant to the prevailing conditions in an abattoir.

Contrary to Campylobacters, which show significant seasonality, there was no seasonality observed for *A. butzleri* in neck skin samples on flock level (see table 6). Seasonal variations in Campylobacter infection in poultry have been reported for several countries (Berndtson, 1996b; Wallace et al., 1997; Nylen et al., 2002) with a peak in summer. Louis et al. (2004) showed that the higher occurrence of Campylobacter is associated with increased temperature, which is consistent with our results.

In poultry meat we found an Arcobacter prevalence of 15.1 % (36/238), which is comparable with other findings. Kabeya et al. (2004) also found 15% (15/100) of the meat samples to be positive for *A. butzleri* by isolating the strains after enrichment and finally confirming the isolates by PCR. A study in Belgium (Houf et al., 2001) describes a prevalence of 24 % (6/25) and the positive samples were detected by direct isolation. De Boer et al. (1996) examined 224 meat samples and isolated 54 *A. butzleri* strains (24%) after enrichment. Three other studies carried out in different countries show higher prevalences (48%-100%) for *A. butzleri* in poultry meat (see table 12). In each study mentioned, a limited number of samples was examined. In all studies *A. butzleri* positive samples were detected by culturing, using various kinds of enrichment broth and culture plates and in some studies a final genus- specific PCR was performed for identification of *A. butzleri*. Only Houf et al. (2001) isolated the strains without a former enrichment step. Though, it is difficult to directly compare results from different studies, due to varying sample sizes and investigation procedures. In a study by Scullion et al. (2003), where three different culturing methods were used for isolation of *Arcobacter* spp. from poultry meat, they obtained different prevalences of *A. butzleri* using the three methods. Houf et al. (2000) examined 14 poultry meat samples by m-PCR after enrichment in two different broths and found two different prevalences for *A. butzleri*.

In feces of cattle, there was no *A. butzleri* found during our study. Literature data on the prevalence of *A. butzleri* in fecal samples of cattle are summarized in table 13. In

a study carried out in Belgium, Van Driessche et al. (2005) isolated *Arcobacter* species from feces of healthy cattle on three unrelated farms by culture method and performing a species-specific multiplex-PCR assay for confirmation. The Arcobacter prevalence on the three farms ranged from 7.5% to 15%. It has to be considered, though, that in this study, positive results included *A. cryaerophilus* and *A. skirrowii*, additional to *A. butzleri.* During that study there was never found an *Arcobacter* species in direct isolation from dairy cows. Only after enrichment, they were able to isolate the three species. With direct isolation they only tested one young cattle positive for *A. cryaerophilus* and one young cattle for *A. skirrowii*. Furthermore, two calves have been detected positive for *A. skirrowii* with direct isolation. *A. butzleri* has never been detected from direct isolates. Only in two cases, *A. butzleri* was detected after enrichment. Öngör et al. (2004) found 7.0% (14/200) of their examined fecal samples to be positive for *A. butzleri* in Turkey. Also in this study, the positive samples for Arcobacter were detected by culturing and using multiplex-PCR performed the identification of the isolates. Kabeya et al. (2003) describe a prevalence of 3.0% (10/332) for *A. butzleri* in feces of cattle in Japan. Positive samples were detected by culturing. After subculturing the colonies, Arcobacter was identified by genus-specific PCR. These results lead to the conclusion that there is a lower prevalence of *A. butzleri* in cattle feces in Switzerland than in Turkey and Japan. Though, it is difficult to directly compare results from different studies, due to varying sample sizes and investigation procedures. This is pointed out in the study of Golla et al. (2002). The objectives of that study were to determine the prevalence of *A. butzleri* in cattle from Texas and to compare the effectiveness levels of various methods in the isolation of this organism. Different kinds of broths and agars were used to culture the samples. The different methods resulted in diversity in results.

In samples of cattle carcasses the prevalence for *A. butzleri* was 19.7% (41/208) in our study. It is not possible to find any comparable data in literature about the prevalence of *A. butzleri* on cattle carcasses. Within cattle we found the same situation as within poultry: A low prevalence in feces and a significant higher prevalence on carcasses. The fact that there was no *A. butzleri* found in feces of cattle shows, that a direct fecal contamination of the carcasses is not likely to take place. It is rather possible that the contamination takes place at different stages of meat processing in the abattoir. This thesis is supported by the finding, that among the strains of cattle, isolated from carcasses, there were only two different genotypes

existing. The six strains originated from samples taken on the same day, but the cattle originated from five different farms. Furthermore, they were not slaughtered directly one after the other, there were gaps of up to 66 animals between the samples taken. This also supports the theory that Arcobacter might, after being carried in by some of the animals, spread in the slaughterhouse and contaminate carcasses.

In minced beef meat, there was no sample found to be positive for *A. butzleri*. Kebaya et al. (2004) described a prevalence of 1,1% (1/90) for *A. butzleri* in beef meat in Japan and Öngör et al. (2004) found 5,2% (5/97) of the beef meat in Turkey to be positive for *A. butzleri* (table 14). Rivas et al. (2003) described a prevalence of 22,0% (7/32) for *A. butzleri* in beef meat in Australia. This is a significant higher prevalence than we found during our study. Their method was similar to our method: After incubating, they detected the *A. butzleri* positive samples by using a multiplex PCR, followed by isolation on blood agar and selective agar.

In pig feces the prevalence for *A. butzleri* was 21.6% (54/250) in our study. Literature data on the prevalence for *A. butzleri* in fecal samples of pigs is summarized in table 15. Van Driessche et al. (2004) designed a study in which Arcobacter species were isolated from clinically healthy pigs from four unrelated farms in Belgium. From the 294 animals examined in total, 31 of them were tested positive for *A. butzleri* after direct isolation. This is a prevalence of 10.5% for *A. butzleri*. There were also seven samples tested positive for *A. cryaerophilus* and three for *A. skirrowii.* They used the same methods as they have used for the study of the cattle samples. Already in 2003, Van Driessche et al. made a study, in which they described the prevalence for *A. butzleri* in pig feces. In that study they found a prevalence of 22.0% (18/82). This result was based on detecting *A. butzleri* by culturing only. In Japan, Kabeya et al. (2003) found a prevalence of 6.0% (15/250) for *A. butzleri* in pig feces. Detection of positive samples was done by culturing. For final identification they used a genus-specific PCR.

Of pig carcasses 19.7% (59/300) were tested positive for *A. butzleri* in our study. It is not possible to find any comparable data in literature on prevalence for *A. butzleri* on pig carcasses. On a single day, the 3rd of January 05, the prevalence for *A. butzleri* on pig carcasses was 68.2% (15/22). On this day, more than a thousand pigs have

been processed in the abattoir, before the pigs, taken the samples from, have been processed.

By genotyping the isolated strains from pig feces and pig carcasses, we detected two different DNA profiles. Profile I was detected on samples of carcasses and feces. Those samples were collected on three individual days. Genotype IV was found on samples of carcasses, which also were collected on three individual days. The fact, that we found the same genotype on different days may indicate, that this organism inhabits the abattoir and contamination occurs also during processing, after *A. butzleri* was brought in and was able to spread in the environment. These findings and the fact that pig carcasses are singed during slaughtering lead to the conclusion that contamination might happen during this processing step.

In pork meat, comparable to cattle meat, there was no sample being tested positive for *A. butzleri* in our study. The two available studies from literature are summarized in table 16. In Japan, 7% (7/100) of pork meat carried *Arcobacter spp.* (Kabeya et al., 2004). 4% (4/100) of the samples carried *A. butzleri*, 3% (3/100) carried *A. cryaerophilus* and 0% (0/100) carried *A. skirrowii*. Their detection method is described above. Rivas et al. (2004) did not only investigated beef meat, they also investigated pig meat samples in the study mentioned above. The prevalence for *A. butzler*i in pork meat was 29.0% (6/21).

To summarize, in our study the prevalence for *A. butzleri* is higher in poultry than in cattle and pig production line. This tendency has also been described in other studies (tables 9 to 15h).

To assess the pathogenicity of *A. butzleri* for humans, evaluation of potential virulence factors is required. However, up to now, little is known about the mechanisms of pathogenicity. Due to the close relation of Arcobacter to Campylobacter and due to a study done by Lee et al. (2002), there are assumptions, that Arcobacter may harbour cytolethal distending toxin (CDT) genes. Cytolethal distending toxins (CDTs), a family of proteins, which interfere with the cell control machinery through their genotoxic activity are produced by multiple pathogens, such as *Escherichia coli* (Toth et al., 2003), *Campylobacter* spp. (Pickett et al., 1996), *Shigella* spp. (Okuda et al., 1995), *Salmonella enterica* serovar Typhi (Haghjoo et al., 2004), *Haemophilus ducreyi* (Cope et al., 1997), *Actinobacillus actinomycetemcomitans* (Mayer et al., 1999), and *Helicobacter* spp. (Chien et al., 2000). CDTs are tripartet toxins encoded by three adjacent or slightly overlapping

genes, *cdt*A, *cdt*B and *cdt*C (Scott et al., 1994). All *cdt* genes are required for arrest of the eukaryotic cell cycle in the G_1 phase and/or the G_2 phase; this arrest characteristically distends cells and eventually causes cell death. Recent studies suggest that within the CDT holotoxin, *Cdt*B is the enzymatically active (A) subunit, which is transported into the nucleus (Lara- Tejero et al., 2000). In the nucleus, CdtB damages through DNase I-like activity host cell DNA, thereby triggering DNA damage checkpoint responses that arrest the cell cycle. The CdtA and CdtC polypeptides constitute the heterodimeric (B) subunit, which is required for CDT binding to target cells and for the intracellular delivery of CdtB (Deng et al., 2003).
We used PCR strategy, based on degenerative primers targeting various regions of *E. coli* and Campylobacter *cdt*B genes to screen our strains. We did not detect the cytolethal distending toxin, which corresponds with the finding of Lee et al. (2002), but contrary to us, they only used Campylobacter specific primers for detection.

Cell adhesion capacity could be another virulence factor. Once a bacterium reaches a host surface, it must adhere to host cells to colonize them. This is particularly important in areas such as small intestine where mucosal surfaces are washed by fluids. In this area, only bacteria that can adhere to mucosal surfaces will be able to stay in the site. Even in relatively stagnant areas such as the colon Brownian motion can move a bacterium that has made contact with a mucosal cell, away from the surface of the cell. Virtually all known bacterial pathogens have some way of attaching themselves firmly to host cells. Two common strategies are used to attach themselves to host cells: pili and afimbrial adesins. Musmanno et al. (1997) found one strain out of 18 isolates of *A. butzleri* from river water samples, which adhered to cells. In our study three strains out of 22 showed adhesion to cells.

In Switzerland, this is the first study reporting prevalence and characterization data of *A. butzleri* isolated from poultry, cattle and pig. The prevalence is higher in poultry meat than in red meat production line. This is comparable to data from literature. Moreover, the further characterization of the strains showed heterogeneity within arcobacters, which may indicate that some strains could potentially be more virulent.

7 Authors' contributions

SK worked on the red meat slaughtering line and retail red meat samples. SR worked on the poultry slaughtering line and retail poultry meat. SK and SR drafted the manuscript. The authors read, commented on and approved of the final manuscript.

8 Tables

Table 1 Most important phenotypical traits for differentiaton between *Arcobacter* spp., *Campylobacter* spp. and *Helicobacter* spp. (according to On, S. L., 1996)

characteristic	Arcobacter	Campylobacter	Helicobacter
aerobic growth at 25°C	+	-	-
Catalase	+	+[1]	+
Oxidase	+	+	+
Urease	-	-[2]	-[3]

[1] *C. consisus* and *C. upsaliensis* are negative
[2] *C. lari* is positive
[3] *H. pylori* is positive

Table 2 Phylogenetic analysis of the 16S rRNA gene sequences of *Arcobacter* spp. strains and strains from closely related genera within the epsilon proteobacteria using the distance matrix tool of the ARB package

	Helicobacter felis (T)	Helicobacter salomonis (T)	Helicobacter pylori (T)	Helicobacter acinonyx (T)	Wolinella succinogenes (T)	Campylobacter hominis (T)	Campylobacter gracilis (T)	Campylobacter rectus (T)	Campylobacter showae (T)	Campylobacter concisus (T)	Campylobacter mucosalis (T)	Campylobacter fetus (T)	Campylobacter lanienae (T)	Campylobacter lari (T)	Campylobacter jejuni (T)	Campylobacter helveticus (T)	Campylobacter upsaliensis (T)	Campylobacter coli (T)	Sulfurospirillum halorespirans	Sulfurospirillum barnesii (T)	Sulfurospirillum barnesii (T)	Sulfurospirillum deleyianum (T	Sulfurospirillum arcachonense	Arcobacter nitrofigilis (T)	Arcobacter skirrowii (T)	Arcobacter cryaerophilus (T)	Arcobacter butzleri
Helicobacter felis (T)	100	99.3	95.7	97.1	90.5	82.8	83	83.5	83.8	85	84.6	84.4	84.4	85.2	84.8	84.8	85.8	84.3	83.5	84.1	83.9	83.8	83.3	83.9	84	84.2	84.8
Helicobacter salomonis (T)	99.3	100	95.3	97	90.1	82.9	82.9	83.1	83.5	84.8	84.6	84.2	84.3	84.9	84.4	84.7	85.8	84.1	83.8	84	84	83.7	83.1	83.7	83.9	84.1	84.5
Helicobacter pylori (T)	95.7	95.3	100	98.1	91.3	83.7	84	84.4	84.6	85.5	84.8	84.9	84.7	85.5	85.4	85.4	85.8	85.3	84	84.6	84.5	84.4	83.3	84	84.8	85.2	85.9
Helicobacter acinonyx (T)	97.1	97	98.1	100	91.2	84.2	83.6	84.3	84.4	85.7	84.8	84.8	84.7	85.8	85.7	85.6	86.2	85.5	84.1	84.7	84.5	84.4	83.9	84.9	85.1	85.5	86.4
Wolinella succinogenes (T)	90.5	90.1	91.3	91.2	100	84.1	85.1	86.2	86.2	86.4	85.8	86	85.6	86.8	86.4	85.8	86	86.4	85.7	86.4	86	86.3	85.2	85.4	85.6	85.8	86.3
Campylobacter hominis (T)	82.8	82.9	83.7	84.2	84.1	100	93.8	93.3	92.7	93	91.2	91.6	90.5	90.1	90.5	88.8	89	90.4	86.6	86.1	85.5	86	85.9	84.2	85.8	86	86
Campylobacter gracilis (T)	83	82.9	84	83.6	85.1	93.8	100	95.6	94.6	94.6	93.8	94	92.4	90.7	90.9	90.3	90.4	91.4	87.5	87	86.6	87.2	86.5	84.7	85.6	86	86.5
Campylobacter rectus (T)	83.5	83.1	84.4	84.3	86.2	93.3	95.6	100	98	95.7	94.3	94.5	92.5	92.3	92.6	91.4	91.6	93.3	87.9	87.2	87	87.6	87	84.7	85	85.5	86
Campylobacter showae (T)	83.8	83.5	84.6	84.4	86.2	92.7	94.6	98	100	94.7	93.5	93.4	91.5	91.2	91.5	90.5	90.6	92.3	87.4	86.8	86.6	87.2	86.8	84.6	85.5	85.9	86
Campylobacter concisus (T)	85	84.8	85.5	85.7	86.4	93	94.6	95.7	94.7	100	96	95.2	94.2	93.6	93.4	91.9	92.1	94.1	88.7	88.4	88	88.4	87.7	87.1	87.5	87.4	88
Campylobacter mucosalis (T)	84.6	84.6	84.8	84.8	85.8	91.2	93.8	94.3	93.5	96	100	97	95.7	93.5	93.7	92.1	93	94.2	88.7	89.1	88.9	89.1	87.5	85.8	86.5	86.8	86.5
Campylobacter fetus (T)	84.4	84.2	84.9	84.8	86	91.6	94	94.5	93.4	95.2	97	100	96.7	94.1	94.3	92.8	93	95.4	88.8	89	88.5	88.8	87.6	85.3	86	86.2	86.2
Campylobacter lanienae (T)	84.4	84.3	84.7	84.7	85.6	90.5	92.4	92.5	91.5	94.6	95.7	96.7	100	95.2	95.3	93.9	93	96.5	89	89.2	88.9	88.8	87.2	85.7	86	86.2	86
Campylobacter lari (T)	85.2	84.9	85.5	85.8	86.8	90.1	90.7	92.3	91.2	93.6	93.5	94.1	95.2	100	98.4	95.9	95.1	97.5	88.4	88.8	88.4	88.4	88.2	86.3	86.5	86.7	86.3
Campylobacter jejuni (T)	84.8	84.4	85.4	85.7	86.4	90.5	90.9	92.6	91.2	93.4	93.7	94.3	95.3	98.4	100	96.6	95.8	98.6	88.1	88.6	88.2	88.2	88.1	86.6	87	87.2	86.7
Campylobacter helveticus (T)	84.8	84.7	85.4	85.6	85.8	88.8	90.3	91.4	90.5	91.9	92.1	92.8	93.9	95.9	96.6	100	98.1	95.7	87.2	87.3	86.9	86.9	86.9	87.2	85.7	85.7	86.1
Campylobacter upsaliensis (T)	85.8	84.5	84.6	85.5	86.4	90.5	92.1	92.5	90.6	92.1	93	93	93	95.1	95.8	98.1	100	94.9	86.6	86.9	86.6	86.8	86.8	85.6	85.6	85.9	86.3
Campylobacter coli (T)	84.3	84.1	85.3	85.5	86.4	90.4	91.4	93.3	92.3	94.1	94.2	95.4	96.5	97.5	98.6	95.7	94.9	100	88.2	88.5	88.1	88.1	88.1	86.1	86.4	86.6	86.3
Sulfurospirillum halorespirans	83.5	83.8	84	84.1	85.7	86.6	87.5	87.9	87.4	88.7	88.7	88.8	89	88.4	88.1	87.2	86.6	88.2	100	97.7	97.1	97.6	92.2	88.4	87.2	87.7	88
Sulfurospirillum barnesii (T)	84.1	84	84.6	84.7	86.4	86.1	87	87.2	86.8	88.4	89.1	89	89.2	88.8	88.6	87.3	86.9	88.5	97.7	100	99.6	98.9	91.8	87.8	87.3	87.8	87.8
Sulfurospirillum barnesii (T)	83.9	84	84.6	84.5	86	85.5	86.6	87	86.6	88	88.9	88.5	88.9	88.4	88.2	86.9	86.6	88.1	97.1	99.6	100	98.6	91.4	87.5	86.9	87.5	87.5
Sulfurospirillum deleyianum (T	83.8	83.7	84.3	84.4	86.3	86	87.2	87.6	87.2	88.4	89.1	88.8	88.8	88.4	88.2	86.9	86.8	88.1	97.6	98.9	98.6	100	92.1	87.9	87.1	87.7	87.9
Sulfurospirillum arcachonense	83.3	83.1	83.3	83.9	85.2	85.9	86.5	87	86.8	87.7	87.5	87.6	87.2	88.2	88.1	87.2	86.8	88.1	92.2	91.8	91.4	92.1	100	87.1	86.9	87.3	87.8
Arcobacter nitrofigilis (T)	83.9	83.1	83.9	84.2	85.4	84.2	84.7	84.7	84.6	87.1	85.8	85.3	86.5	86.3	86.7	86.1	86.8	86.1	88.4	87.8	87.5	87.9	87.1	100	94.3	94.2	94.5
Arcobacter skirrowii (T)	84	83.9	84.8	85.1	85.6	85.8	85.6	85	85.5	87.5	86.5	86	86	86.5	87	85.7	85.6	86.4	87.2	87.3	86.9	87.1	86.9	94.3	100	98.6	97.1
Arcobacter cryaerophilus (T)	84.2	84.1	85.2	85.5	85.8	86	86	85.5	85.9	87.4	86.8	86.2	86.2	86.7	87.2	85.9	85.9	86.6	87.7	87.8	87.5	87.7	87.3	94.2	98.6	100	97.4
Arcobacter butzleri	84.8	84.5	85.9	86.4	86.3	86	86.5	86	86	88	86.5	86.2	86	86.3	86.7	86.1	86.3	86.3	88	87.8	87.5	87.9	87.8	94.5	97.1	97.4	100

Table 3 Prevalence of Arcobacter isolation from retail raw meats

Country	origin	Arcobacter positive samples (%)*	Literature
USA	chicken meat	84.0	Johnson and Murano 1999
Spain	chicken meat	53.0	Gonzalez et al., 2000
Mexico	chicken meat	40.0	Villarruel-Lopez et al., 2003
Japan	chicken meat	49.0	Morita et al., 2004
Japan	chicken meat	23.0	Kabeya et al., 2004
Thailand	chicken meat	100.0	Morita et al., 2004
Australia	chicken meat	73.0	Rivas et al., 2004
US	pork meat	32.0	Ohlendorf and Murano 2002
Mexico	pork meat	51.5	Villarruel-Lopez et al., 2003
Japan	pork meat	7.0	Kabeya et al., 2004
Australia	pork meat	29.0	Rivas et al., 2004
Mexico	beef meat	28.8	Villarruel-Lopez et al., 2003
Japan	beef meat	2.2	Kabeya et al., 2004
Australia	beef meat	2.0	Rivas et al., 2004

*in all studies cultural isolation methods were used

Table 4 Prevalence of *A. butzleri* in poultry, cattle and pig samples

	feces	carcass	meat
Poultry	1.4% (15/1090)	53.6% (133/248)[1)]	15.1% (36/238)
		64.7% (134/207)[2)]	
Cattle	0.0 % (0/210)	19.7% (41/208)	0.0% (0/150)
Pig	21.6% (54/250)	19.7% (59/300)	0.0 % (0/52)

[1)] neck skin samples taken after plucking

[2)] skin samples taken from further processing stages of carcasses

Table 5 Prevalence of *C. jejuni* and *C. coli* in poultry samples

	feces	carcass	meat
C.jejuni	14.9% (164/1099)	20.9% (51/244)[1)]	7.7% (17/220)
		6.9% (14/204)[2)]	
C.coli	0.5% (5/1099)	0.0% (0/244) [1)]	0.0% (0/220)
		0.0% (0/204) [2)]	

[1)] neck skin samples taken after plucking

[2)] skin samples taken from further processing stages of carcasses

Table 6 Seasonality of Arcobacter and Campylobacter isolation prevalence on flock level

	Arcobacter		Campylobacter	
	feces	neck skin	feces	neck skin
Nov	0.0% (0/20)	45.0% (9/20)	35.0% (7/20)	20.0% (4/20)
Dec	14.7% (5/34)	50.0% (17/34)	8.8% (3/34)	11.8% (4/34)
Jan	0.0% (0/44)	45.5% (20/44)	29.5% (13/44)	13.6% (7/44)
Feb	6.7% (3/45)	55.8% (24/43)	17.5% (7/40)	7.9% (3/38)
March	2.9% (1/35)	37.9% (11/29)	25.7% (9/31)	29.6% (8/27)
April	0.0% (0/28)	54.2% (13/24)	21.4% (6/28)	37.5% (9/24)
Mai	2.8% (1/36)	72.2% (26/36)	30.3% (10/33)	33.3% (11/33)
June	0.0% (0/24)	54.2% (13/24)	37.5% (9/24)	33.3% (8/24)

Table 7 In vitro activity of ciprofloxacin, erythromycin and tetracyclin against *A. butzleri* strains isolated form poultry, pig and cattle.

species	strain	ciprofloxacin MIC (mg/l)	erythromycin MIC (mg/l)	tetracyclin MIC (mg/l)
poultry	AHH37	0,125	3,0	2,0
	AHH41	0,185	1,0	1,5
	AHH42	0,094	1,5	0,75
	AHH43	0,125	2.0	1,0
	AHH172	0,064	0,19	0,125
	AH157	0,25	1,5	2,0
	AH165	0,064	3,0	1,5
cattle	HR199	0.064	1,5	1,5
	HR201	0,064	1,5	1,5
	HR203	0,064	1,0	1,5
	HR204	0,064	1,5	1,0
	HR205	0,064	2,0	1,5
	HR208	0,125	2,0	2,0
pig	HS22	0,094	4,0	3,0
	HS25	0,094	1,5	1,75
	HS33	0,094	2,0	1,0
	HS50	0,084	3,0	1,0
	HS56	0,094	3,0	2,0
	HS63	0,064	2,0	1,0
	HS122	0,25	3,0	2,0
	HS125	0,125	3,0	2,0
	S165	0,19	6,0	2,0

AHH: poultry neck skin; AH: poultry carcass; HR; cattle carcass; HS: pig carcass, S: pig feces

Table 8 Genotyping results of the 22 *A. butzleri* strains isolated from poultry, pig and cattle.

Strain	origin	pattern
HS25	pig	I
HS22	pig	I
HS125	pig	I
HS122	pig	I
S165	pig	I
AHH41	poultry	II
AHH37	poultry	II
AHH43	poultry	III
AHH42	poultry	III
HS63	pig	IV
HS33	pig	IV
HS56	pig	IV
HS50	pig	IV
AHH172	poultry	V
AH157	poultry	VI
AH165	poultry	VII
HR199	cattle	VIII
HR201	cattle	VIII
HR203	cattle	VIII
HR204	cattle	VIII
HR205	cattle	VIII
HR208	cattle	IX

HS: pig carcass; S: pig feces; AHH: poultry neck skin, AH: poultry carcass; HR: cattle carcass

Table 9 Results of the adhesion assay on HEP-2 cells

species	strain	adhesion assay
poultry	AHH37	++
	AHH41	+++
	AHH42	-
	AHH43	+
	AHH172	-
	AH157	-
	AH165	-
cattle	HR199	-
	HR201	-
	HR203	-
	HR204	-
	HR205	-
	HR208	-
pig	HS22	-
	HS25	-
	HS33	-
	HS50	-
	HS56	-
	HS63	-
	HS122	-
	HS125	-
	S165	-

AHH: poultry neck skin poultry; AH: poultry skin; HR: cattle carcass; HS: pig carcass; S: pig feces; + - +++: intensity of adhesion

Table 10 Prevalence of *A.butzleri* positive samples in poultry feces

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Belgium	480	0 (0.0)	Houf et al., 2002
Japan	234	16 (6.8)	Kabeya et al., 2003
UK	2	0 (0.0)	Gude et al., 2005
USA	407	4 (1.0)	Wesley and Baetz 1999

Table 11 Prevalence of *A.butzleri* positive samples on poultry carcasses

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Belgium	45 [1)]	16 (36.0)	Houf et al., 2001
Canada	125 [2)]	121 (97.0)	Lammerding et al., 1996
France	201 [2)]	161 (80.0)	Festy et al., 1993
UK	25 [2)]	25(100.0)	Atabay et al., 1998
UK	2 [1)]	2(100.0)	Gude et al., 2005
Turkey	75 [2)]	49 (65.0)	Atabay et al., 2003

1) neck skin after plucking

2) carcasses from abattoir and/or retail markets

Table 12 Prevalence of *A.butzleri* positive samples in poultry meat

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Australia	22	16 (73.0)	Rivas et al., 2004
Belgium	25	6 (24.0)	Houf et al., 2001
Japan	100	15 (15.0)	Kabeya et al., 2004
Japan	41	20 (48.0)	Morita et al., 2004
Netherlands	224	53 (24.0)	De Boer et al., 1996
Thailand	10	10(100.0)	Morita et al., 2004

Table 13 Prevalence of *A.butzleri* positive samples in bovine feces

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Belgium	51	2 (3.9)	Van Driessche et al., 2003
Japan	332	10 (3.0)	Kabeya et al., 2003
Belgium	276	0 (0.0)	Van Driessche et al., 2005
Turkey	200	14 (7.0)	Öngör et al., 2004
USA	200	18 (9.0)	Golla et al., 2002

Table 14 Prevalence of *A. butzleri* positive samples in bovine meat

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Australia	32	7 (22.0)	Rivas et al., 2003
Japan	90	1 (1.1)	Kabeya et al., 2004
Turkey	97	5 (5.2)	Öngör et al., 2004

Table 15 Prevalence of *A.butzleri* positive samples in pig feces

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Belgium	294	31 (10.5)	Van Driessche et al., 2004
Belgium	82	18 (22.0)	Van Driessche et al., 2003
Japan	250	15 (6.0)	Kabeya et al., 2003

Table 16 Prevalence of *A. butzleri* positive samples in pig meat

Country	No. of samples	Arcobacter positive samples n (%)	Literature
Australia	21	6 (29.0)	Rivas et al., 2004
Japan	100	4 (4.0)	Kabeya et al., 2004

9 Figures

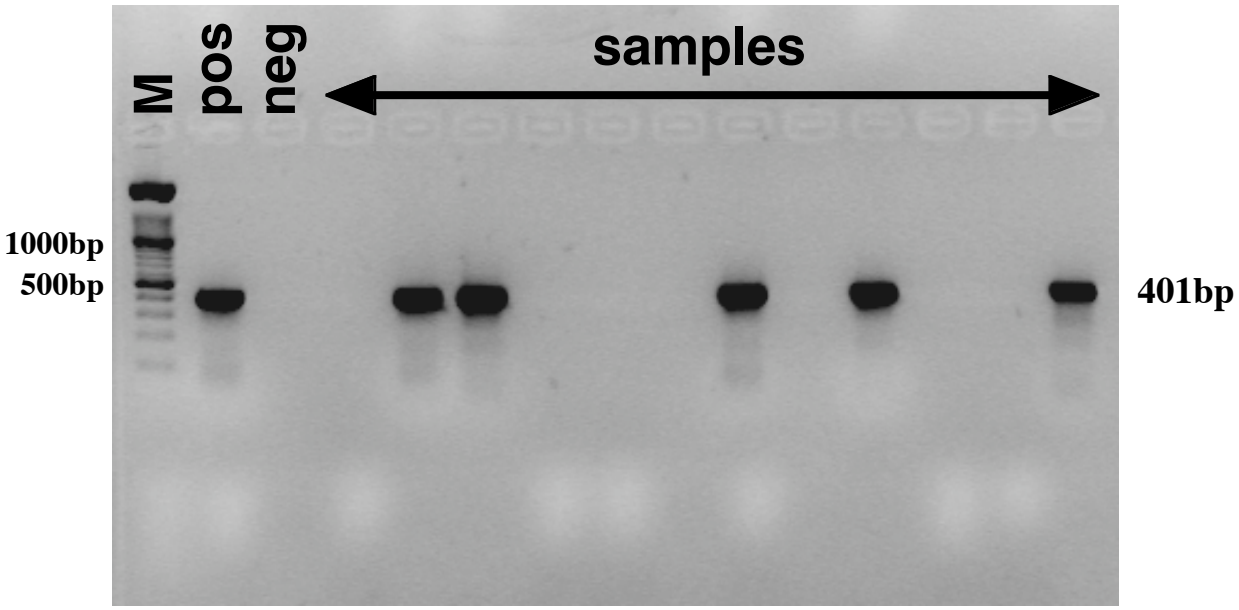

Figure 1. Representative *A. butzleri* PCR products with positiv and negativ control. M: 100bp molecular weight marker, pos: positiv control; neg: negativ control; samples: PCR products from various samples

```
                1                                                   50
ATCC49616       ACAGGTGCTG CACGGCTGTC GTCAGCTCGT GTCGTGAGAT GTTGGGTTAA
AF140           ACAGGTGCTG CACGGCTGTC GTCAGCTCGT GTCGTGAGAT GTTGGGTTAA
AH107           ACAGGTGCTG CACGGCTGTC GTCAGCTCGT GTCGTGAGAT GTTGGGTTAA
AH103           ACAGGTGCTG CACGGCTGTC GTCAGCTCGT GTCGTGAGAT GTTGGGTTAA

               51                                                  100
ATCC49616       GTCCCGCAAC GAGCGCAACC CTCGTCCTTA GTTGCTAACA GTTCGGCTGA
AF140           GTCCCGCAAC GAGCGCAACC CTCGTCCTTA GTTGCTAACA GTTCGGCTGA
AH107           GTCCCGCAAC GAGCGCAACC CTCGTCCTTA GTTGCTAACA GTTCGGCTGA
AH103           GTCCCGCAAC GAGCGCAACC CTCGTCCTTA GTTGCTAACA GTTCGGCTGA

               101                                                 150
ATCC49616       GAACTCTAAG GAGACTGCCT ACGCAAGTAG GAGGAAGGTG AGGATGACGT
AF140           GAACTCTAAG GAGACTGCCT ACGCAAGTAG GAGGAAGGTG AGGATGACGT
AH107           GAACTCTAAG GAGACTGCCT ACGCAAGTAG GAGGAAGGTG AGGATGACGT
AH103           GAACTCTAAG GAGACTGCCT ACGCAAGTAG GAGGAAGGTG AGGATGACGT

               151                                                 200
ATCC49616       CAAGTCATCA TGGCCCTTAC GTCCAGGGCT ACACACGTGC TACAATGGGG
AF140           CAAGTCATCA TGGCCCTTAC GTCCAGGGCT ACACACGTGC TACAATGGGG
AH107           CAAGTCATCA TGGCCCTTAC GTCCAGGGCT ACACACGTGC TACAATGGGG
AH103           CAAGTCATCA TGGCCCTTAC GTCCAGGGCT ACACACGTGC TACAATGGGG

               201                                                 250
ATCC49616       TATACAAAGA GCAGCAATAC GGTGACGTGG AGCAAATCTC AAAAATGCCT
AF140           TATACAAAGA GCAGCAATAC GGTGACGTGG AGCAAATCTC AAAAATGCCT
AH107           TATACAAAGA GCAGCAATAC GGTGACGTGG AGCAAATCTC AAAAATGCCT
AH103           TATACAAAGA GCAGCAATAC GGTGACGTGG AGCAAATCTC AAAAATGCCT

               251                                                 300
ATCC49616       CCCAGTTCGG ATTGTAGTCT GCAACTCGAC TACATGAAGT TGGAATCGCT
AF140           CCCAGTTCGG ATTGTAGTCT GCAACTCGAC TACATGAAGT TGGAATCGCT
AH107           CCCAGTTCGG ATTGTAGTCT GCAACTCGAC TACATGAAGT TGGAATCGCT
AH103           CCCAGTTCGG ATTGTAGTCT GCAACTCGAC TACATGAAGT TGGAATCGCT

               301                 330
ATCC49616       AGTAATCGTA GATCAGCTAT GCTACGG
AF140           AGTAATCGTA GATCAGCTAT GCTACGG
AH107           AGTAATCGTA GATCAGCTAT GCTACGG
AH103           AGTAATCGTA GATCAGCTAT GCTACGG
```

Figure 2 Alignment of *A. butzleri* reference strain (ATCC 49616) 330 bp region (nt. 977 – 1303) against the sequence of PCR products amplified from the corresponding region of *A. butzleri* isolates.

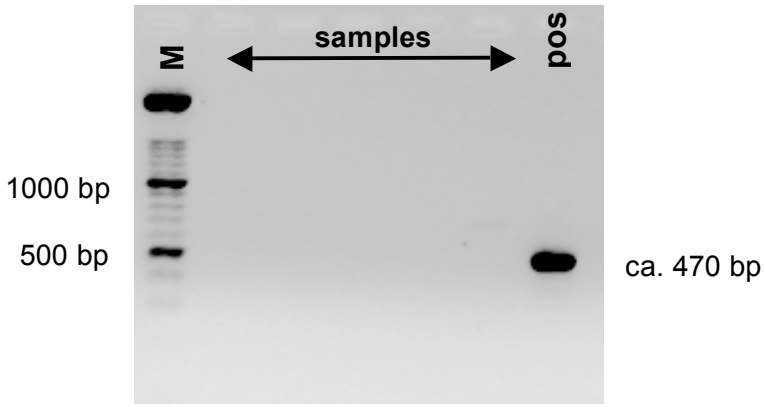

Figure 3. CDT-Toxin (*cdt*B) PCR of *A. butzleri* with positive control. M: 100bp molecular weight marker, pos: Campylobacter positive control; samples: isolated *A. butzleri* strains.

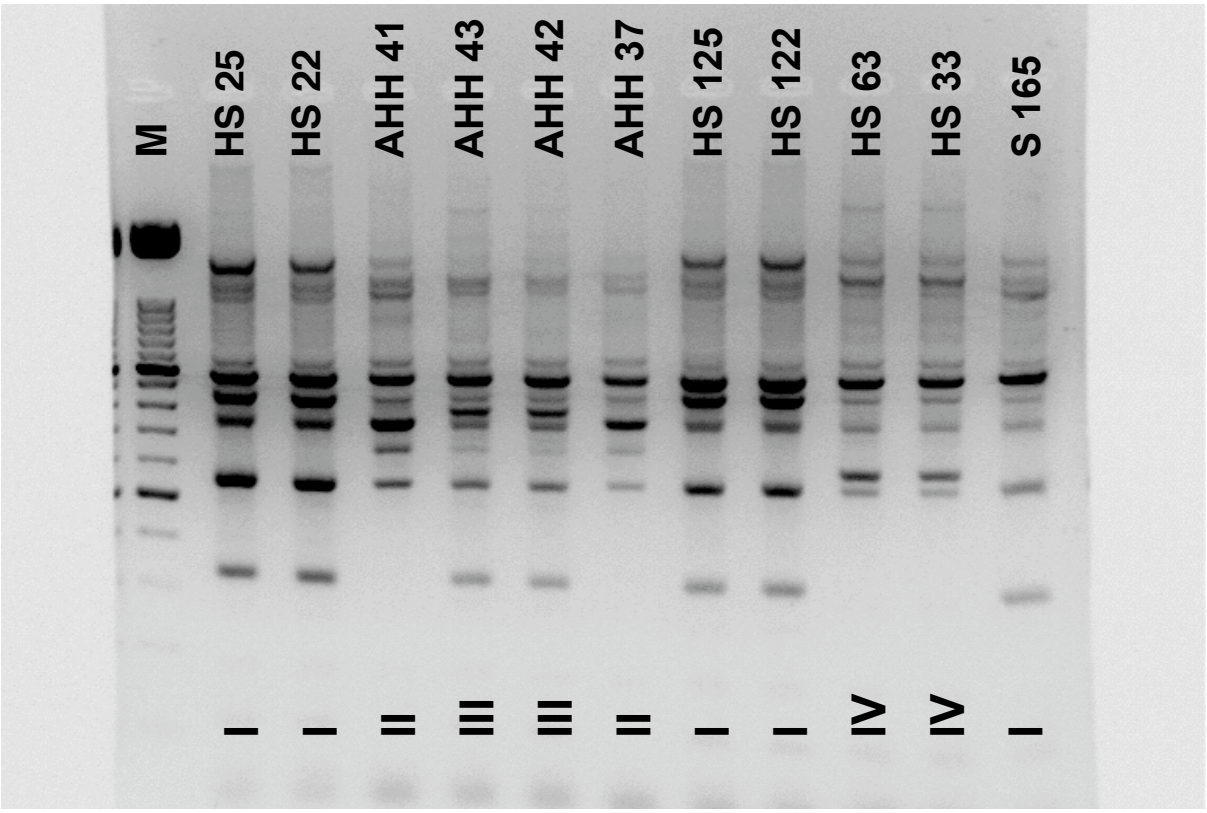

Figure 4. ERIC- PCR fingerprinting patterns of *A. butzleri* isolates from various samples. M: 100bp molecular weight marker; HS: pig skin; AHH: poultry neck skin; S: pig feces; I-IV: different genotypes found

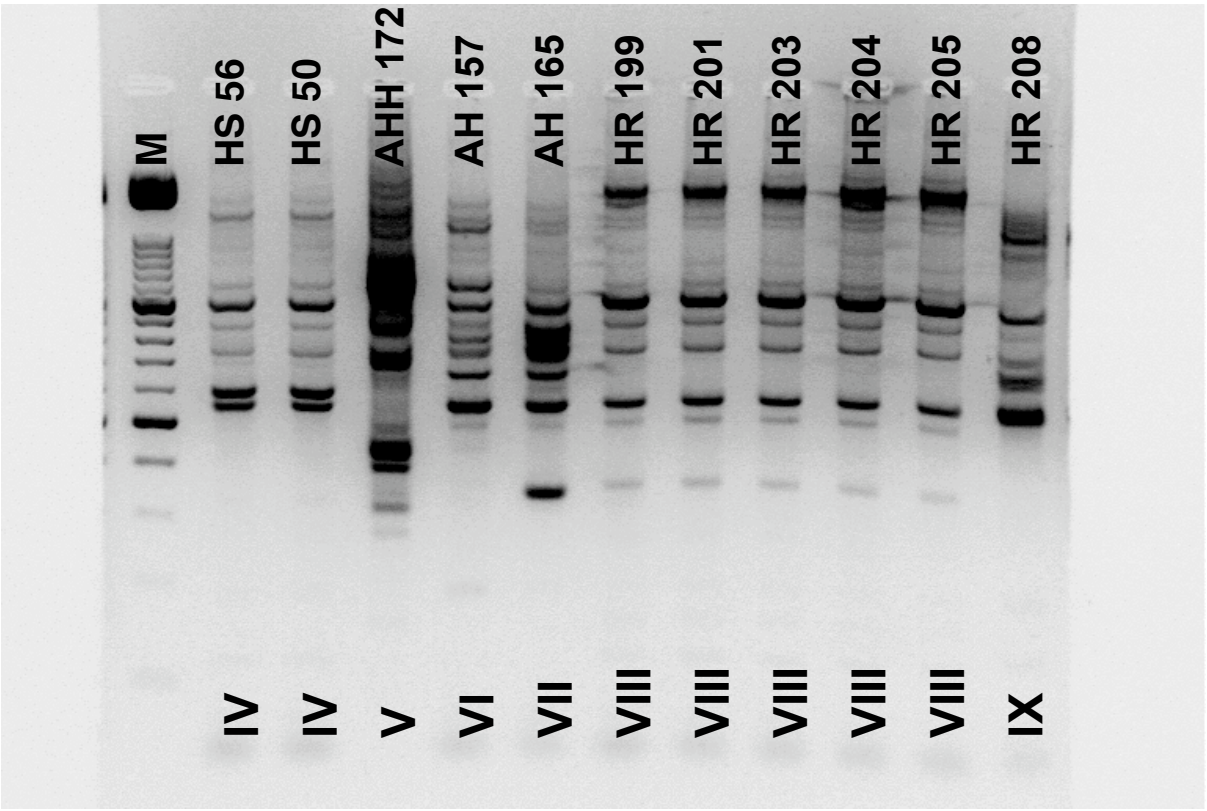

Figure 5. ERIC- PCR fingerprinting patterns of *A. butzleri* isolates from various samples. M: 100bp molecular weight marker; HS: pig skin; AHH: poultry neck skin; AH: poultry skin; HR: skin cattle; V-IX: different genotypes found

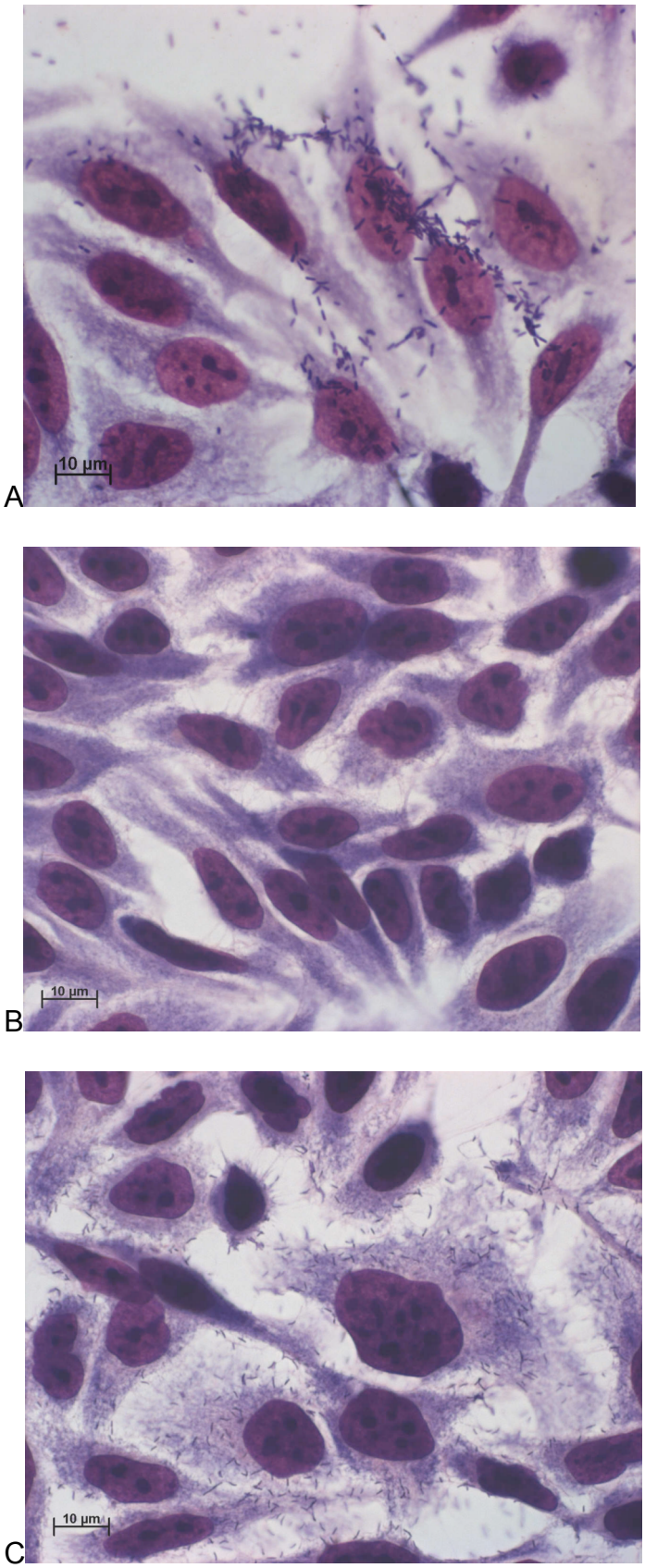

Figure 6. Adhesion assay on HEp-2 cells (3h assay); A: positive control *E. sakazakii* strain ES5; B: *A. butzleri* strain HR 201; C: *A. butzleri* strain AHH 41

10 References

Al Rashid, S.T., Dakuna, I., Louie H., D., Vandamme, P., Johnson, W., Chan, V.L., 2000. Identification of *Campylobacter jejuni, C. coli, C. lari, C. upsaliensis*, *Arcobacter butzleri*, and *A. butzleri*-like species based on the *gly*A gene. Journal of Clinical Microbiology 38, 1488-1494.

Anderson, K.F., Kiehlbauch, J.A., Anderson, D.C., McClure, H.M., Wachsmuth, I.K., 1993. *Arcobacter (Campylobacter) butzleri*-associated diarrhea illness in a nonhuman primate population. Infection and Immunity 61, 2220-2223.

Anonymous, 1996. International Commission on Microbiological Specifications for Food. Campylobacter. In: Microorganisms in Food, 5. Characteristics of Microbial Pathogens pp. 45-65. Blackie Academic & Professional. London.

Assanta, M.A., Roy, D., Lemay, M.J., Montpetit, D., 2002. Attachment of *Arcobacter butzleri*, a new waterborne pathogen, to water distribution pipe surfaces. Journal of Food Protection 65, 1240-1247.

Atabay, H.I., Corry, J.E., 1997. The prevalence of campylobacters and arcobacters in broiler chickens. Journal of Applied Microbiology 83, 619-626.

Atabay, H.I., Corry, J.E., On, S.L., 1998. Diversity and prevalence of *Arcobacter* spp. in broiler chickens. Journal of Applied Microbiology 84, 1007-1016.

Atabay, H.I., Aydin, F., 2001. Susceptibility of *Arcobacter butzleri* isolates to 23 antimicrobial agents. Letters in Applied Microbiology.

Atabay, H.I., Bang, D.D., Aydin, F., Erdogan, H.M., Madsen, M., 2002. Discrimination of *Arcobacter butzleri* isolates by polymerase chain reaction-mediated DNA fingerprinting. Letters in Applied Microbiology 35, 141-145.

Atabay, H.I., Aydin, F., Houf, K., Sahin, M., Vandamme, P., 2003. The prevalence of *Arcobacter* spp. on chicken carcasses sold in retail markets in Turkey and identification of the isolates using SDS-PAGE. International Journal of Food Microbiology 25, 21-28.

Beach, J.C., Murano, E.A., Acuff, G.R., 2002. Prevalence of Salmonella and Campylobacter in beef cattle from transport to slaughter. Journal of Food Protection 65, 1687-1693.

Berndston, E., Emanuelson, U., Engvall, A. Danielsson-Tham, M.L., 1996b. A 1-year epidemiological study of campylobacters in 18 swedish chicken farms. Preventive Veterinary Medicine 26, 167-185.

Bonardi, S., Maggi, E., Pizzin, G., Morabito, S., Caprioli, A., 2001. Faecal carriage of Verocytotoxin-producing *Escherichia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. International Journal of Food Microbiology 66, 47-53.

Burnens, A.P., Schaad, U.B., Nicolet, J., 1992. Isolation of *Arcobacter butzerli* from a gil with gastro-enteritis on Yersinia CIN agar. Medical Microbiology Letters 1, 251-256.

Chien, C.C., Taylor, N.S., Ge, Z., Schauer, D.B., Young, V.B., Fox, J.G., 2000. Identification of *cdt*B homologues and cytolethal distending toxin activity in enterohepatic *Helicobacter* spp. Journal of Medical Microbiology 49, 525-534.

Cope, L.D., Lumbley, S., Latimer, J.L., Klesney-Tait, J., Stevens, M.K., Johnson, L.S., Purven, M., Munson, R.S., Hansen, E.J., 1997. A diffusible cytotoxin of *Hamophilus ducrey*. Proceedings of the National Academy of Sciences USA 94, 4056-4061.

Corry, J.E., Atabay, H.I., 2001. Poultry as a source of Campylobacter and related organism. Journal of Applied Microbiological symptoms.

Corry, J.E., Atabay, H.I., Forsythe, S.J., Mansfield, L.P., 2003. Culture media for the isolation of Campylobacters, Helicobacters and Arcobacters. In: Handbook of culture media for food microbiology: progress in industrial microbiology, pp. 271-317, Corry, J.E., Curtis, Baird, R.M. (Eds.), vol. 37, 2nd edition, Elsevier, Amsterdam.

Deng, K., Hansen, J.E., 2003. A CdtA-CdtC complex can block killing of HeLa cells by *Haemophilus ducreyi* cytolethal distending toxin. Infectious Immunology 71, 6633-6640.

D'Sa, E. M., Harrison, M. A., 2004. Effect of pH, NaCl Content and Temperature on Growth and Survival of *Arcobacter* spp. Journal of Food Protection, 68, 18-25.

Donachie, S.P., Bowman, J.P., On, S.L., Alam, M., 2005. *Arcobacter halophilus* sp. nov., the first obligate halophile in the genus Arcobacter. Journal of Systematic Evolutionary Microbiology 55, 1271-7.

Eifert, J.D., Castle, R.M., Pierson, F.W., Larsen, C.T., Hackney, C.R., 2003. Comparison of sampling techniques for detection of *Arcobacter butzleri* from chickens. Poultry Science 82, 1898-1902.

Engberg, J., On, S.L., Harrington, C.S., Gerner-Smidt, P., 2000. Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter* and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. Journal of Clinical Microbiology 38, 286-291.

Fera, M.T., Maugeri, T.L., Giannone, M., Gugliandolo, C., La Camera, E., Blandino, G., Carbone, M., 2003. In vitro susceptibility of *Arcobacter butzleri* and *Arcobacter cryaerophilus* to different antimicrobial agents. International Journal of Antimicrobial Agents 21, 488-491.

Fera, M.T., Maugeri, T.L., Gugliandolo, C., Beninati, C., Giannone, M., La Camera, E., Carbone, M., 2004. Detection of *Arcobacter* spp. in the coastal environment of the mediterranean sea. Applied and Environmental Microbiology 70, 1271-1276.

Festy, B., Squinazi, F., Marin, M., Derimay, R., Lior, H. 1993. Poultry meat and water as the possible sources of *A.butzleri* associated with human disease in Paris, France. Acta Gastroenterologica Belgium.

Golla, S.C., Murano, E.A., Johnson, L.G., Tipton, N.C., Cureington, E.A., Savell, J.W., 2002. Determination of the occurrence of *Arcobacter butzleri* in beef and dairy cattle from Texas by various isolation methods. Journal of Food Protection 65, 1849-1853.

Gonzàlez, I., Garcia, T., Antolin, A., Hernandez, P.E., Martin, R., 2000. Development of a combined PCR-culture technique for the rapid detection of *Arcobacter* spp. in chicken meat. Letters in Applied Microbiology 30, 207-212.

Gude, A., Hillman, T.J., Helps, C.R., Allen, V.M., Corry, J.E., 2005. Ecology of *Arcobacter* species in chicken rearing and processing. Letters in Applied Microbiology 41, 82-87.

Haghhjoo, E., Galan, J.E., 2004. Salmonella typhi encodes a functional cytolethal distending toxin that is delivered into host cells by a bacterial-internalization pathway. Proceedings of the National Academy of Sciences USA 101, 4614-4619.

Harmon, K.M., Wesley, I.V., 1996. Identification of Arcobacter isolates by PCR. Letters in Applied Microbiology 23, 241-244.

Harmon, K.M., Wesley, I.V., 1997. Multiplex PCR for the identification of Arcobacter and differentiation of *Arcobacter butzleri* from other arcobacters. Veterinary Microbiology 58, 215-227.

Harrass, B., Schwarz, S., Wenzel, S., 1998. Identification and characterization of Arcobacter isolates from broilers by biochemical tests, antimicrobial resistance patterns and plasmid analysis. Journal of Veterinary Medicine 45, 87-94.

Harvey, R.B., Anderson, R.C., Young, C.R., Hume, M.E., Genovese, K.J., Ziprin, R.L., Farrington, L.A., Stanker, L.H., Nisbet, D.J., 1999. Prevalence of

Campylobacter, *Salmonella* and *Arcobacter* species at slaughter in market age pigs. Advanced Experimental Medicine Biology 473, 237-239.

Hilton, C.L., Mackey, B.M., Hargreaves, A.J., Forsythe, S.J., 2001. The recovery of *Arcobacter butzleri* NCTC 12481 from various temperature treatments. Journal of Applied Microbiology 91, 929-932.

Houf, K., Tutenel, A., De Zutter, L., Van Hoof, J., Vandamme, P., 2000. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiology Letters 193, 89-94.

Houf, K., Devriese, L.A., De Zutter, L., Van Hoof, J., Vandamme, P., 2001. Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products.

Houf, K., De Zutter, L., Van Hoof, J., Vandamme, P., 2002. Assessment of the genetic diversity among Arcobacters isolated from poultry products by using two PCR-based typing methods. Applied and Environmental Microbiology 68, 2172-2178.

Houf, K., De Zutter, L., Van Hoof, J., Vandamme, P., 2002. Occurrence and distribution of *Arcobacter* species in poultry processing. Journal of Food Protection 65, 1233-1239.

Houf, K., De Zutter, L., Verbeke, B., Van Hoof, J., Vandamme, P., 2003. Molecular characterization of Arcobacter isolates collected in a poultry slaughterhouse. Journal of Food Protection 66, 364-369.

Houf, K., Devriese, L.A., Haesebrouck, F., Vandenberg, O., Butzler, J.P., van Hoof, J., Vandamme, P., 2004. Antimicrobial susceptibility patterns of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from humans and broilers. Mircobial drug resistance 10(3), 243-247.

Houf, K., On, S.L., Coenye, T., Mast, J., Van Hoof, J., Vandamme, P., 2005. *Arcobacter cibarius* sp. nov. isolated from broiler carcasses. International Journal of Evolutionary Microbiology 55, 713-717.

Hume, M.E., Harvey, R.B., Stanker, L.H., Droleskey, R.E., Poole, T.L., Zhang, H.B., 2001. Genotypic variation among Arcobacter isolates from a furrow-to-finish swine facility. Journal of Food Protection 64, 645-651.

Hurtado, A., Owen, R.J., 1997. A molecular scheme based on 23S rRNA gene polymorphisms for rapid identification of *Campylobacter* and *Arcobacter* species. Journal of Clinical Microbiology 35, 2401-2404.

Jacob, J., Woodward, D., Feuerpfeil, I., Johnson, W.M., 1998. Isolation of *Arcobacter butzleri* in raw water and drinking water treatment plants in Germany. Zentralblatt für Hygiene und Umweltmedizin 201, 189-198.

Jacobs-Reitsma, W., 2000. Campylobacter in the food supply. In: Campylobacter, pp. 467-482, Nachamkin, I., Blaser, M.,J., (eds.), 2nd edition, ASM Press, Washington, DC.

Johnson, L.G., Murano, E.A., 1999. Comparison of three protocols for the isolation of *Arcobacter* from poultry. Journal of Food Protection 62, 610-614.

Johnson, L.G., Murano, E.A., 2002. Lack of a cytolethal distending toxin among Arcobacter isolates from various sources. Journal of Food Protection 65, 1789-1795.

Kabeya, H. Maruyama, S., Morita, Y., Kubo, M., Yamamoto, K., Arai, S., Izumi, T., Kobayashi, Y., Katsube, Y., Mikami, T., 2003. Distribution of *Arcobacter* species among livestock in Japan. Veterinary Microbiology 93, 153-158.

Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., Abe, M., Katsube, Y., Mikami, T., 2004. Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. International Journal of Food Microbiology 90, 303-308.

Karch, H., Böhm, H., Schmidt, H., Gunzer, F., Aleksic, S., Heesemann, J., 1993. Clonal structure and pathogenicity of shiga-like toxin-producing, sorbitol-fermenting *Escherichia coli* O157:H-. Journal of Clinical Microbiology 31, 1200-1205.

Keener, K. M., Bashor, M. P., Curtis, P. A., Sheldon, B. W., Kathariou, S., 2004. Comprehensive review of Campylobacter and poultry processing. The Institute of Food Technologists, Comparative Reviews in Food Science in Food Safety 3, 105-116.

Kiehlbauch, J.A., Brenner, D.J., Nicholson, M.A., Baker, C.N., Patton, C.M., Steigerwalt, A.G., Wachsmuth, I.K., 1991. *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrhea illness. Journal of Clinical Microbiology 29, 376-385.

Kielbauch, J.A., Baker C.N., Wachsmuth, I.K., 1992. In vitro susceptibilities of aerotolerant Campylobacter isolates to 22 antimicrobial agents. Antimicrobial Agents and Chemotherapy 36, 717-722.

Koeuth, T., Versalovic, J., Lupski, J.R., (no year) Differential subsequence conservation supports the mosaic nature of interspersed repetitive BOX elements in bacteria. Submitted for publication.

Lammerding, A.M., Harris, J.E., Lior, H., Woodward, D.E., Cole, L., Muckle, C.A., 1996. Isolation method for recovery of Arcobacter butzleri from fresh poultry and poultry products. In: Campylobacters, Helicobacters and Related Organisms. Proceedings of the 18th International Workshop on Campylobacters, Helicobacters and Related Organisms, pp 530-531, Newell, D.G., Ketley, J., Feldmann, R.A. (Eds.), Plenum Publishing Corporation, New York.

Lara- Tejero, M., Galan, J.E., 2000. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. Sciene 290, 354-357.

Lau, S.K., Woo, P.C., Teng, J.L., Leung, K.W. Yuen, K.Y., 2002. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient with acute gangrenous appendicitis. Molecular Pathology 55, 182-185.

Lerner, J., Brumberger, V., Preac-Mursic, V., 1994. Severe diarrhoea associated with *A. butzleri.* European Journal of Clinical Microbiology and Infectious Disease 13, 660-662.

Mansfiled, L.P., Forsythe, S.J., 2000. *Arcobacter butzleri*, *A. skirrowii* and *A. cryaerophilus*- potential emerging human pathogens. News in Medical Microbiology 11, 161-170.

Marshall, S.M., Selito, P.L., Woodward, D.L., Johnson, W.M., Rodgers, F.G., Mulvey, M.R., 1999. Rapid identification of Campylobacter, Arcobacter, and Helicobacter isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA level. Journal of Clinical Microbiology 37, 4158-4160.

Martin, B., Humbert, O., Camara, M., Guenzi, E., Walker, J., Mitchell, T., Andrew P., Prudhomme, G., Alloing, G., Hakenbeck, R., Morrison, D.A., Boulnois G.J., Claverys J.P., 1992. A highly conserved repeated DNA element located in the chromosome of *Streptococcus pneumoniae*. Nucleic Acids Research 20, 3479-3483.

Mayer, M.P., Bueno, L., Hansen, E.J., DiRienzo, J.M., 1999. Identification of a cytolethal distending toxin gene locus and features of a virulence- associated region in *Actinobacillus actinomycetemcomitans*. Infectious Immunology 67, 1227-1237.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., Tauxe R. V., 1999. Food-related illness and death in the United States. Emerging Infection Diseases 5, 607-625.

Moreno, Y., Botella, S., Alonso, J.L., Ferrus, M.A., Hernandez, M., Hernandez, J., 2003. Specific detection of Arcobacter and Campylobacter strains in water and sewage by PCR and fluorescent in situ hybridisation. Applied and Environmental Microbiology 69, 1181-1186.

Morita, Y., Maruyama, S., Kabeya, H., Boonmar, S., Nimsuphan, B., Nagai, A., Kozawa, K., Nakajima, T., Mikami, T., Kimura, H., 2004. Isolation and phylogenetic analysis of *Arcobacter* spp. in ground chicken meat and environmental water in Japan and Thailand. Microbiology and Immunology 48, 527-533.

Musmanno, R.A., Russi, M., Lior, H., Figura, N., 1997. In vitro virulence factors of *Arcobacter butzleri* strains isolated from superficial water samples. New Microbiology 20, 63-68.

Nylen, G., Dunstan, F., Palmer, S., Andersson, Y., Bager, F., Cowden, J., Feierl, G., Galloway, Y., Kapperud, G., Megraud, F., Molbak, K., Peterson, L., Ruutu, P., 2002. The seasonal distribution of Campylobacter infection in nine European countries and New Zealand. Epidemiological infections 128, 383-390

Ohlendorf, D.S., Murano, E.A., 2002. Prevalence of *Arcobacter* spp. in raw ground pork from several geographical regions according to various isolation methods. Journal of Food Protection 65, 1700-1705.

Okuda, J., Kurazono, H., Takeda, Y., 1995. Distribution of the cytolethal distending toxin A gene (*cdt*A) among species of Shigella and Vibrio, and cloning and sequencing of the cdt gene from *Shigella dysenteriae*. Microbial and Pathogenesis 18, 167- 172.

On, S.L., Stacey, A., Smyth, J., 1995. Isolation of *Arcobacter butzleri* from neonate with bacteraemia. The Journal of Infections 31, 225-227.

On, S.L., 1996. Identification methods for Campylobacters, Helicobacters, and related organisms. Clinical Microbiology Reviews 9, 405-422.

On, S.L., Jensen, T.K., Bille-Hansen, V., Jorsal, S.E., Vandamme, P., 2002. Prevalence and diversity of A*rcobacter* spp. isolated from the internal organs of spontaneous porcine abortions in Denmark. Veterinary Microbiology 85, 159-167.

On, S.L., Harrington, C.S., Atabay, H.I., 2003. Differentiation of *Arcobacter* species by numerical analysis of AFLP profiles and description of a novel Arcobacter from pig abortions and turkey faeces. Journal of Applied Microbiology 95, 1096-1105.

Öngör, H., Cetinkaya, B., Acik, M.N., Atabay, H.I., 2004. Investigation of Arcobacters in meat and faecal samples of clinically healthy cattle in Turkey. Letters in Applied Microbiology 38, 339-344.

Phillips, C.A., 1999. The effect of citric acid, lactic acid, sodium citrate and sodium lactate, alone and in combination with nisin, on the growth of *Arcobacter butzleri*. Letters in Applied Microbiology 29, 424-428.

Phillips, C.A., Duggan, J., 2001. The effect of EDTA and trisodium phosphate, alone and in combination with nisin, on the growth of *Arcobacter butzleri* in culture. International Journal of Food Microbiology 18, 547-554.

Phillips, C.A., 2001. *Arcobacter* spp. in food: isolation, identification and control. Trends in Food Science &Technology 12, 263-275

Pickett, C.L., Pesci, E.C., Cottle, D.L., Russel, G., Erdem, A.N., Zeytin, H., 1996. Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* spp. *cdt*B gene. Infectious Immunology 63, 2070-2078.

Rice, E.W., Rodgers, M.R., Wesley, I.V., Johnson, C.H., Tanner, S.A., 1999. Isolation of *Arcobacter butzleri* from ground water. Letters in Applied Microbiology 28, 31-35.

Ring, M., Zychowska, A., Stephan, R., 2005. An epidemiological study on Campylobacter poultry flock colonization in 14 broiler flocks. Avian Diseases, in press.

Rivas, L., Fegan, N., Vanderlinde, P., 2004. Isolation and characterization of *Arcobacter butzleri* from meat. International Journal of Food Microbiology 91, 31-41.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular cloning: a laboratory manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Scullion, R., Harrington, C.S., Madden R.H., 2004. A comparison of three methods for the isolation of *Arcobacter* spp. from retail raw poultry meat in Northern Ireland. Journal of Food Protection 67, 799-804.

Schroeder-Tucker, L., Wesley, I.V., Kiehlbauch, J.A., Larson, D.J., Thomas, L.A., Erickson, G.A., 1996. Phenotypic and ribosomal RNA characterization of *Arcobacter* species isolated from porcine aborted fetuses. Journal of Veterinary Diagnostic Investigation 8, 186-195.

Scott, D.A., Kaper, J.B., 1994. Cloning and sequencing of the genes encoding *Escherichia coli* cytolethal distending toxin. Infectious Immunology 62, 244-251.

Skirrow, M.B., 1994. Diseases due to Campylobacter, Helicobacter and related bacteria. Journal of Comparative Pathology 111, 113-149.

Smitherman, R.E., Genigeorgis, C.A., Farver, T.B., 1984. Preliminary observation on the occurrence of Campylobacter jejuni at four California chicken ranches. Journal of Food Protection 47, 293-298.

Stampi, S., De Luca, G., Varoli, O., Zanetti, F., 1999. Occurrence, removal and seasonal variation of thermophilic Campylobacters and Arcobacter in sewage sludge. Zentralblatt für Hygiene und Umweltmedizin 202, 19-27.

Toth, I., Herault, F., Beutin, L., Oswald, E., 2003. Production of cytolethal distending toxins by pathogenic *Escherichia coli* isolated from human and animal sources: establishment of the existence of a new *cdt* variant (type IV). Journal of Clinical Microbiology 41, 4285-4291.

Vandamme, P., De Ley, J., 1991. Proposal for a new family Campylobacteraceae. International Journal of Systematic Bacteriology 41, 451-455.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tygat, R., De Ley, J., 1991. Revision of Campylobacter, Helicobacter and Wolinella taxonomy: emendation of generic description and proposal for Arcobacter gen. nov. International Journal of Systematic Bacteriology 41, 88-103.

Vandamme, P., Giesendorf, B.A., Van Belkum, A., Periard, D., Lauwers, S., Kersters, K., Butzler, J.P., Goossens, H., Quint, W.G., 1992a. Discrimination of epidemic and sporadic isolates of *Arcobacter butzleri* by polymerase chain reaction-mediated DNA fingerprinting. Journal of Clinical Microbiology 31, 3317-3319.

Vandamme, P., Pugina, P., Benzi, G., van Etterijk, R., Vlases, L., Dersters, K., Butzler, J.P., Lior, H., Lauwers, S., 1992. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. Journal of Clinical Microbiology 30, 2335-2337.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G., Butzler, J.P., Vandamme, P., 2004. *Arcobacter* species in humans. Emerging Infectious Diseases, Vol. 10, No. 10.

Van Driessche, E., Houf, K., Van Hoof, J., De Zutter, L., Vandamme, P., 2003. Isolation of *Arcobacter* species from animal feces. FEMS Microbiology Letters 229, 243-248.

Van Driessche, E., Houf, K., Vangroenweghe, F., Nollet, N., De Zutter, ,L., Vandamme, P., Van Hoof J., 2004. Occurrence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs. Research in Microbiology 155, 662-666

Van, Driessche, E., Houf, K., De Zutter, L., Van Hoof, J., 2005. Prevalence, enumeration and strain variation of *Arcobacter* species of healthy cattle in Belgium. Veterinary Microbiology 105, 149-154.

Versalovic, J.T., Koeuth, T., Lupski, J.R., 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Research 19, 6823-6831.

Villarruel-Lopez, A., Marquez-Gonzales, M., Garay-Martinez, L.E., Zepeda, H., Castillo, A., Mota de la Garza, L., Murano, E.A., Torres-Vitela, R., 2003. Isolation of *Arcobacter* spp. from retail meats and cytotoxic effects of isolates against vero cells. Journal of Food Protection 66, 1374-1378.

Wallace, J.S., Stanley, K.N., Currie, J.E., Diggle, P.J., Jones, K., 1997. Seasonality of thermophilic Campylobacter populations in chickens. Journal of Applied Mircobiology 82, 219-224.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M., Siddique, I., 2000. Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. Applied and Environmental Microbiology 66, 1994-2000.

Winters, D.K., Slavik, M.F., 2000. Multiplex PCR detection of *Campylobacter jejuni* and *Arcobacter butzleri* in food products. Molecular Cell Probes 14, 95-99.

Wirsen, C.O., Sievert, S.M., Cavanaugh, C.M., Molyneaux, S.J., Ahmad, A., Taylor, L.T., DeLong, E.F., Taylor, C.D., 2002. Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* spp. that produces filamentous sulfur. Applied and Environmental Microbiology 68, 316-325.

Wybo, I., Breynaert, J., Lauwers, S., Lindenburg, F., Houf, K., 2004. Isolation of *Arcobacter skirrowii* from a patient with chronic diarrhea. Journal of Clinical Microbiology 42, 1851-1852.

Yamano, R., Ohara, M., Nishikubo, S., Fujiwara, T., Kawamoto, T., Ueno, Y., Komatsuzawa, H., Okuda, K., Kurihara, H., Suginaka, H., Oswald, E., Tanne, K., Sugai, M., 2003. Prevalence of cytolethal distending toxin production in periodontopathogenic bacteria. Journal of Clinical Microbiology 42, 1391-1398.

Yan, J.J., Ko, W.C., Huang, A.H., Chen, H.M., Jin, Y.T., Wu, J.J., 2000. *Arcobacter butzleri* bacteremia in a patient with liver cirrhosis. Journal of the Formosian Medical Association 99, 166-169.

11 Acknowledgments

We would like to express our gratitude to everyone, who contributed to this work.

Special thanks go to:

Prof. Dr. Roger Stephan, Institute for Food Safety and Hygiene ,Vetsuisse Faculty University of Zurich, for the expert advice and assistence on proofreading and editing this dissertation.

Prof. Dr. Kurt Houf, Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Merelbeke, Belgium for accepting to referee our dissertation.

Dr. Taurai Tasara, Mrs. Sandra Schuhmacher and Jean-Philippe Mange for their assistance and scientific support on *A. butzleri* PCR and the adhesion assay.

Mr. J. Giletycz und Mrs. U. Baumgartner for the production of the culture mediums.

The staff members of the Institute for Food Safety and Hygiene for their assistance on the laboratory work.

Dr. Margrit Stamm and staff members of the Bell Poulet AG for working with us and taking the poultry samples.

The staff members of the slaughterhouse Zurich for their friendly acquaintance and assistance on sampling.

Curriculum vitae

Name	Sibille, Keller
Geburtsdatum	11. Juni 1976
Geburtsort	Zug
Nationalität	Schweiz
Heimatort	Meggen, LU

1983 - 1989	Primarschule in Meggen
1989 - 1996	Gymnasium an der Kantonsschule in Luzern
1996	Matura Typus B
1996 - 1997	Zwischenjahr mit Sprachaufenthalt in London, Certificate in Advanced English
1997 - 1999	Studium der Veterinärmedizin an der Universität Bern, Schweiz
1999 - 2003	Studium der Veterinärmedizin an der Universität Zürich, Schweiz
2004	Staatsexamen an der Universität Zürich, Schweiz
2004 - 2005	Doktorandin, Institut für Lebensmittelsicherheit und -hygiene der Vetsuisse-Fakultät Universität Zürich, Zürich, Schweiz

31. August 2005

Curriculum vitae

Name	Sibylle, Räber
Geburtsdatum	08. Januar 1977
Geburtsort	Uster
Nationalität	Schweiz
Heimatort	Benzenschwil AG

1984 - 1990	Primarschule in Wetzikon
1990 - 1997	Kantonsschule Zürcher Oberland in Wetzikon
1997	Matura Typus B
1997 - 2002	Studium der Veterinärmedizin an der Universität Zürich, Schweiz
1999	Austauschsemester an der École Vétérinaire Maisons- Alfort, Paris
2002	Staatsexamen an der Universität Zürich, Schweiz
2003 - 2004	Assistenztierärztin, Tierarztpraxis von Dr. Men Bischoff, Sent, Schweiz
2004	Assistenztierärztin (Vertretung), Gemischtpraxis Dammbühl AG von Pius Spescha, Wängi, Schweiz
2004 - 2005	Doktorandin, Institut für Lebensmittelsicherheit und -hygiene der Vetsuisse-Fakultät Universität Zürich, Zürich, Schweiz
2005	Assistenztierärztin (Vertretung), Tierarztpraxis von Dr. Martin Glanzmann, Oberhasli, Schweiz

31. August 2005